EXHIBIT A

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

UNITED THERAPEUTICS CORPORATION,)
)
----------Plaintiff,)
) Case No.
vs.) 23-CV-975-RGA
)
LIQUIDIA TECHNOLOGIES, INC.,)
)
----------Defendant.)

TRANSCRIPT OF PRETRIAL CONFERENCE

PRETRIAL CONFERENCE had before the Honorable Richard G. Andrews, U.S.D.C.J., in Courtroom 6A on the 30th of May, 2025.

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THE COURT: Good morning. Please be seated. I guess we should start with Mr. Flynn. You don't have to tell me everybody who's here, but why don't you tell me the people you expect might have speaking roles.

MR. FLYNN: Good morning, Your Honor. Michael Flynn from Morris Nichols on behalf of United Therapeutics. At counsel table is William Jackson from Goodwin Procter, Doug Carsten from McDermott, Will & Emery; Jake Vallen from McDermott, Will & Emery; and Shaun Snader, who's in-house counsel at UTC. Others that I expect to have a speaking role are Adam Burrowbridge and Lillian Spetrino from McDermott, Will & Emery and Eric Romeo and Gabriel Ferrante from Goodwin Procter.

THE COURT: Thank you, Mr. Flynn.

And I see Mr. Hoeschen out there. I guess I see -- yes, and I see Ms. Keller too.

Mr. Hoeschen.

MR. HOESCHEN: Good morning, Your Honor. Nathan Hoeschen from Shaw Keller on behalf of Defendant Liquidia. With me at counsel table from Cooley LLP I have Sanya Sukduang, Jon Davies, Dan Knauss, and Phil Morton. And the next row we have Rachel Preston, John Habibi, and Robert Minn.

THE COURT: And, Mr. Hoeschen, there's asterisks next to the first four people. Is that because they're

special?

MR. HOESCHEN: I believe they're who will be speaking today.

MR. SUKDUANG: The court reporter asked us to identify who will be speaking today.

THE REPORTER: It's my fault.

THE COURT: First time I've seen asterisks.

All right. So everybody in here is associated with one side or the other? There's no independent individuals? Okay.

(The following proceedings were held under and seal:)

THE COURT: So we're under seal for just a minute. One of the things that I'm curious about is I saw in the papers that -- reference to Liquidia could launch when they get FDA approval or something like that, and I'm wondering, is that -- is there any information that that's on the horizon?

MR. SUKDUANG: Sanya Sukduang from Cooley on behalf of Liquidia.

Yes, on Friday, May 23, last Friday, Liquidia received final FDA approval for Yutrepia and we notified, actually, the Middle District of North Carolina because on May 9, UTC sued Liquidia in the Middle District of North Carolina on a new patent that issued back in June, July of

2022. It's in the same family as the invalidated '793 patent. In that case, they moved for a TRO and PI. That was argued May 20 Tuesday in front of Judge Schroeder and the parties are awaiting a decision from Judge Schroeder on UTC's TRO PI.

THE COURT: And the communication you got from the FDA on May 23, does that mean that, essentially, if there's no legal impediment from the court, you could have launched on the 24th? Or is that when you noticed that it's coming and --

MR. SUKDUANG: No, it's not. We could launch immediately on the 23rd when we received it. All -- to date, all regulatory and legal impediments have been removed but for adjudication of this case and the TRO PI.

THE COURT: But this case, you're not under any restraints from this case.

MR. SUKDUANG: No, we're not. As of right now, there are no impediments.

THE COURT: In North Carolina, the judge there -- while this motion is pending, you could, if you want, launch. There's no sort of interim "don't do anything until I decide this"?

MR. SUKDUANG: Correct. We can launch. There's no stopping. UTC filed for a TRO and he hasn't decided that yet.

THE COURT: Okay. All right. Thank you.

MR. SUKDUANG: You're welcome.

THE COURT: In North Carolina, are the parties represented by the same counsel?

MR. JACKSON: Yes, Your Honor. Mr. Sukduang and I were both the ones arguing that North Carolina TRO a week and a half ago, whenever it was.

THE COURT: All right. Thank you.

So I saw the letter filed last night that said -- that elected what the invalidity and unenforceability defenses are. Am I correct that the written description is basically only directed to three of the six claims?

MR. SUKDUANG: Again, Sanya Sukduang, Your Honor. At this point, it's directed to a single claim, Claim 9. As written in the reports, it covered several claims, but because they dropped, it's Claim 9.

THE COURT: All right. As long as we're on that tack, the public sale, does that cover all the claims?

MR. SUKDUANG: It will cover all claims.

THE COURT: All right. And the theory there is that device Tyvaso -- can you explain the --

MR. SUKDUANG: Yes, absolutely. As of 2009, Tyvaso, the commercial product, was on sale. The Tyvaso labeling has certain dosing requirements. Those dosing

requirements were --

First, Tyvaso was sold. There's no dispute on Tyvaso being sold.

Second, and this goes to the ready-for-patenting issue on public sale, the Tyvaso labeling has initial dosing and maintenance dosing requirements. Those dosing requirements were used by multiple physicians across the country to treat PH-ILD patients and improve their exercise capacity. So prior sale of the elements of Claim 1. Dr. Rothblatt confirmed that in her 2018 --

THE COURT: I think I know what the evidence is.

MR. SUKDUANG: With respect to the dependent claims, it's inherent in that the -- what you'll hear at trial is the claims of the '327 patent are the increased trial.

THE COURT: Right. I sort of have gotten that, is Plaintiff's experts say that increase trial proves all the things that are necessary for the dependent claims.

MR. SUKDUANG: Correct. So it's inherent there. So Claim 1 and 17 -- 17 is a dependent claim directed to walking distance. 1 and 17 would be literal and 5, 6, and 9 inherency. 14 comes under obviousness because it's a dry powder claim.

THE COURT: So the public sale as anticipation doesn't apply to 14?

MR. SUKDUANG: Would not apply to 14.

THE COURT: Okay. You were going to say?

MR. SUKDUANG: 14 is -- depends from 11. 11 is the pulsed inhalation device. 14 is -- we think it's seven claims identified.

THE COURT: I'm satisfied by six.

MR. SUKDUANG: So 14 is the dry powder. And Tyvaso is inhalation, not a dry powder.

THE COURT: All right. Hold on a minute. Thank you. Thank you. You can sit down.

MR. JACKSON: Your Honor, on that last point, can I be heard?

THE COURT: Yeah.

MR. JACKSON: On the prior -- we took your order the other day to heart. We had a meet-and-confer last night and we talked through a bunch of things that are mooted out and what the claims cover.

THE COURT: It may not surprise you I've been trying to figure out the same thing but with less information. So more information is useful.

MR. JACKSON: During our meet-and-confer last night, we agreed that prior sale is just under 102(a)(1). Their second defense, which is anticipation by the increase trial protocol, we confirmed what exhibit that is. I think that's Exhibit 8, DTX 8. We confirmed the scope of

obviousness in view of Faria-Urbina includes the Faria-Urbina and supplement thereto that they say is part of the same article.

Written description they said is only for Claim 9.

THE COURT: All right. Yes.

MR. JACKSON: They are no longer contesting priority, the priority date of the patent.

THE COURT: That was on my list because I was thinking that based on, certainly, the first election they had that it seems like that was a moot issue too because public sale seemed the theory. I can see why it's moot on that too.

MR. JACKSON: Yes.

With respect to the various Dauberts and motions in limine --

THE COURT: Hold on just a second. I can't write as fast as you can talk.

Go ahead.

MR. JACKSON: UTC's Daubert regarding Dr. Hill, which is the DTX 284, 285. I'm sorry. DI 284, 285.

THE COURT: Yes.

MR. JACKSON: And the response is 299.

THE COURT: Okay.

MR. JACKSON: And the reply is 313. That's

moot.

THE COURT: And Dr. Hill, there were two issues but they're both moot; right?

MR. JACKSON: So the Daubert regarding Dr. Hill is moot. There's a motion in limine that touches on some of the things he said. Some of that is moot. Some of it isn't, but the Daubert itself, correct.

THE COURT: There were -- and possibly I've got the motion in limine and Daubert reversed, but there was one that involved Dr. Hill and I think Dr. Channick. They were the same thing. I guess that was a motion in limine.

In any event, I understand Dr. Hill, in terms of -- hold on just a minute. I do really mean hold on a minute because I forgot to bring my motions in limine with me. I will be right back. Don't go anywhere.

I'm sorry. For Dr. Hill, you said the Daubert was 285?

MR. JACKSON: 284 is the cover motion and 285 is the memo.

THE COURT: Hold on one moment here. Okay. Got it. All right. So Dr. Hill, 284 is moot; yes? And that's because he was about inequitable conduct; right?

MR. JACKSON: We only moved to exclude certain portions of his opinions, like he had a couple-hundred-page report and we moved to exclude 20 or so pages, and that was

about inequitable conduct. Inequitable conduct is no longer in the case, so that motion is done.

THE COURT: That's good. So let's just make sure that we get that in. Docket Item 284, which is the Daubert motion related to Dr. Hill, is dismissed as moot. All right. Go ahead.

MR. JACKSON: The Daubert with regard to Dr. Wertheim, the motion itself is 280, memo is 281. That's also moot.

THE COURT: I'm glad that's moot. Motion in limine in Docket Item 280, that's dismissed as moot.

MR. JACKSON: I think that is it. There are aspects of paragraphs in -- of their motions in limine that are no longer relevant because those are out, but I don't think I have to carve those out for you.

THE COURT: I'm hopeful before we're finished today I will have ruled on all the motions in limine and all the Daubert motions, but that's helpful to have that. That's two less I have to recall when I'm thinking about these things.

MR. JACKSON: And to confirm what I think you and Mr. Sukduang discussed, the prior sale -- their prior sale anticipation defense applies to five of the six claims but does not apply to 14.

THE COURT: Right. Even though I think what I

understood Mr. Sukduang to be saying was that it applies to 14 through an obviousness defense of some sort.

MR. JACKSON: I don't doubt they have -- I think their defense to 14 is their obviousness in view of Faria-Urbina.

THE COURT: Okay. Is that what you meant, Mr. Sukduang?

MR. SUKDUANG: Yes.

THE COURT: All right. That's good to know. All right. Anything else?

MR. JACKSON: I think that -- I'm just trying to help Your Honor if Your Honor has questions. I just figured clarifying that would be helpful.

THE COURT: When we get around to discussing these, you can tell me if I start to opine on things that are no longer relevant. Cut me off. Okay? All right. Thank you, Mr. Jackson.

MR. JACKSON: Thank you, sir.

THE COURT: Maybe it makes sense to actually try to go through the rest of these motions because in terms of the pretrial order itself, other than the time limits and -- there was really no actual disputes that I saw.

MR. SUKDUANG: There's one correction to be made from the parties' meet-and-confer. Specifically, last night during the meet-and-confer, the parties forgot to put in

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that UTC has secondary considerations to bring commercial success, I think copying maybe unexpected results. So the parties agreed that Liquidia would provide its rebuttal to objective indicia after UTC presents whatever objective indicia.

THE COURT: So basically the agreement is UTC infringement, you all noninfringement and your invalidity defenses, UTC secondary consideration, you rebuttal to secondary consideration.

MR. SUKDUANG: Correct. The parties are going to submit a revised pretrial cover just to reflect that so it's clear.

THE COURT: Okay. Well, all right. That will be fine. I think it should be clear now, too, but it's good to have it written down someplace that's easily accessible.

All right. So we've got these Dauberts. And the first one is Docket Item 278, which is the motion to exclude the opinion and testimony of Dr. Thisted, and I think this boils down to whether or not he can testify from the perspective of a POSA that's what the issue is here; right?

MR. KNAUSS: Yes, Your Honor.

THE COURT: So the people we know, Mr. Sukduang he comes up here every time and says who he is. The people we don't know, not so much. So anybody who's not

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Mr. Sukduang or Mr. Jackson ought to identify themselves when they're speaking. Okay.

So yes, this is about Dr. Thisted and whether he testified from the perspective of a POSA. Are you, Plaintiff, seriously contesting, seriously asserting that he's a POSA?

MR. BURROWBRIDGE: Adam Burrowbridge on behalf of the plaintiff. Your Honor, our position is that Dr. Thisted has expertise within the scope of the POSA definition. Both parties agree that --

THE COURT: He's a well-qualified biostatistician and he teaches or taught in medical school so he knows something about medicine, but he's certainly not the POSA that's envisioned by either of your descriptions; right?

MR. BURROWBRIDGE: Well, Your Honor, we would respectfully disagree with respect to the fact that he has a Ph.D. and experience with drug development, which is part of UTC's proposed definition.

THE COURT: There's also the part about two years of treating, which he does not have; right?

MR. BURROWBRIDGE: He does not have that portion of the definition. Case law permits POSAs to rely on others in the field.

THE COURT: That would permit your doctor to

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rely on Dr. Thisted, but it doesn't work the other way around.

MR. BURROWBRIDGE: Both parties agree that the POSA would have access to teammates.

(Cross-talk).

THE COURT: So Dr. Thisted can testify about stuff that biostatisticians testify about and he can certainly testify he teaches first-year medical students, but then it's up to whoever -- and I forgot whose expert is who -- but it's your medical doctor is the one who's going to be offering opinions on infringement and invalidity, not Dr. Thisted.

MR. BURROWBRIDGE: Correct, Your Honor. I'd like to make two points in response.

First, under *SEB versus Montgomery Ward*, the Federal Circuit has found that experts with an adequate relationship of their expertise to the claimed invention can opine from the perspective of a POSA. So we have that authority.

Second, what we want to preserve is the ability for Dr. Thisted to critique and rebut the experts on the other side that are opining on what a POSA would think. Here, Dr. Thisted is well-positioned to disagree with that because both parties' POSA standards have a biostatistics training built in because they both require M.D.s and M.D.s

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have biostatistics training. So what we would like is for Dr. Thisted be able to take the stand and say a POSA would disagree with that because a POSA would have some baseline understanding of how statistics work and how clinical design is important and be able to critique that opinion.

THE COURT: Why can't your medical doctor say that?

MR. BURROWBRIDGE: Our medical doctor says that as well.

THE COURT: So then you're going up against the second thing, which is generally you get one person to say what's infringing and what's invalid, not multiple people. So is there -- the thing that I was curious about is, is there any reason why your medical doctors can't be the infringement invalidity -- they've offered opinions on invalidity and infringement and presumably to the extent it's all biostatistics, they're either relying on what Dr. Thisted said or they're relying on their own knowledge of biostatistics; right?

MR. BURROWBRIDGE: That's correct, Your Honor, and I think our approach was to preserve the ability to critique the POSA's opinion from the other side.

THE COURT: If one of the POSAs or the defendant says something like -- starts spewing bad biostatistical analysis, I think you've got the right to have, assuming

EXHIBIT B

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

UNITED THERAPEUTICS CORPORATION, Plaintiff, v. LIQUIDIA TECHNOLOGIES, INC., Defendant.	C.A. No. 1:23-cv-00975-RGA **HIGHLY CONFIDENTIAL**

DEFENDANT LIQUIDIA TECHNOLOGIES, INC.'S SECOND AMENDED INVALIDITY CONTENTIONS

Co. v. Teva Pharms. Int'l GmbH, 8 F.4th 1331, 1344 (Fed. Cir. 2021); *see also Hoffman-La Roche Inc. v. Apotex Inc.*, 748 F.3d 1326, 1331 (Fed. Cir. 2014) ("Conclusive proof of efficacy is not necessary to show obviousness. All that is required is a reasonable expectation of success.").

A. Asserted Claims 1–11 and 14–19 of the '327 Patent Are Invalid for Obviousness-Type Double Patenting over the Claims of the '793 Patent

Asserted Claims 1-11 and 14-19 of the '327 patent are invalid for obviousness-type double patenting over the claims '793 patent, which is assigned to UTC. "Obviousness-type double patenting is a judge-made doctrine that prevents an extension of the patent right beyond the statutory time limit. It requires rejection of an application claim when the claimed subject matter is not patentably distinct from the subject matter claimed in a commonly owned patent." *In re Berg*, 140 F.3d 1428, 1431–32 (Fed. Cir. 1988). If the claims at issue are not patentably distinct from the earlier reference claims, the claims at issue are invalid. *Sun Pharm. Industries, Ltd. v. Eli Lilly and Co.*, 611 F.3d 1381, 1384–85 (Fed. Cir. 2010). Obviousness-type double patenting applies because the '327 and '793 patents are commonly owned by UTC and the claims of the '327 patent are not patentably distinct from those of the earlier-expiring, and invalid, '793 patent. Moreover, the '793 patent is in a different patent family so the safe harbor provision pursuant to 35 U.S.C. § 121 does not apply, and UTC has not filed a terminal disclaimer for the '327 patent disclaiming the portion of the patent term beyond the expiration of the '793 patent. This deficiency cannot be cured by filing a terminal disclaimer, because the '793 patent has been ruled invalid.

1. Claim 1 of the '327 Patent is Invalid for Obviousness-type Double Patenting Over the '793 Patent

Asserted Claim 1 discloses "A method of improving exercise capacity in a patient having pulmonary hypertension associated with interstitial lung disease, comprising administering by inhalation to the patient having pulmonary hypertension associated with interstitial lung disease an effective amount of at least 15 micrograms up to a maximum tolerated dose of treprostinil or a

Contentions. Discovery and Liquidia's investigation are ongoing, and Liquidia reserves the right to modify and/or supplement its First Amended Invalidity Contentions.

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Dated: October 30, 2024

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CERTIFICATE OF SERVICE

I certify that I caused copies of the foregoing document to be served on October 30, 2024 upon the following in the manner indicated:

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EXHIBIT C

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Paper 10
Entered: June 5, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

SANOFI PASTEUR INC. AND SK CHEMICALS CO., LTD.,
Petitioner,

v.

PFIZER INC.,
Patent Owner.

Case IPR2018-00188
Patent 9,492,559 B2

Before TONI R. SCHEINER, JEFFREY N. FREDMAN, and JACQUELINE T. HARLOW, *Administrative Patent Judges.*

FREDMAN, *Administrative Patent Judge*.

DECISION
Denying Institution of *Inter Partes* Review
35 U.S.C. § 314(a)

I. INTRODUCTION

A. Background

Sanofi Pasteur Inc. and SK Chemicals Co., Ltd. ("Petitioner") filed a Petition (Paper 3, "Pet.") requesting an *inter partes* review of claims 1–45 (the "challenged claims") of U.S. Patent No. 9,492,559 B2 (Ex. 1001, "the '559 patent") that claims benefit of priority to U.S. Provisional 61/929,547 (Ex. 1002, "the '547 provisional"). *See* 35 U.S.C. §§ 311–319. Pfizer Inc. ("Patent Owner") filed a Preliminary Response. Paper 8 ("Prelim. Resp."). The Board, acting on behalf of the Director, has jurisdiction under 35 U.S.C. § 314.

For the reasons that follow, the Board determines that the prior art relied upon by Petitioner is excluded because the '559 patent receives benefit of priority to the '547 provisional and, further, that the cited references do not qualify as prior art under AIA 35 U.S.C. § 102(a)(2). Therefore, the Board declines to institute an *inter partes* review.

B. Related Proceedings

Petitioner indicates that a concurrent Petition for *inter partes* review of the '559 patent was filed (IPR2018-00187) and that several IPRs were filed by a different petitioner (IPR2017-02131, IPR2017-02132, IPR2017-02136, IPR2017-02138). Pet. 2.

C. The '559 Patent (Ex. 1001)

The '559 patent "relates to vaccination of human subjects, in particular infants and elderly, against pneumoccocal infections. . . ." Ex. 1001, 1:21–22. "Pneumonia, febrile bacteraemia and meningitis are the most common manifestations of invasive pneumococcal disease, whereas

bacterial spread within the respiratory tract may result in middle-ear infection, sinusitis or recurrent bronchitis." *Id.* at 1:28–32.

The '559 patent teaches the "etiological agent of pneumococcal diseases, *Streptococcus pneumoniae* (pneumococcus), is a Gram-positive encapsulated coccus,[1] surrounded by a polysaccharide capsule.[2] Differences in the composition of this capsule permit serological differentiation between about 91 capsular types." *Id.* at 1:49–53. "Pneumococcal conjugate vaccines (PCVs) are pneumococcal vaccines used to protect against disease caused by *S. pneumoniae* (pneumococcus)." *Id.* at 1:59–61. "There are currently three PCV vaccines available on the global market: PREVNAR® (called PREVENAR® in some countries) (heptavalent[3] vaccine), SYNFLORIX® (a decavalent vaccine) and PREVNAR 13® (tridecavalent vaccine)." *Id*. at 1:61–65.

The '559 patent teaches "there is a need to address remaining unmet medical need for coverage of pneumococcal disease due to serotypes not found in PREVNAR 13® and potential for serotype replacement over time." *Id*. at 2:3–6.

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[1] A "coccus" is defined as "a spherical bacterium." *See Coccus Definition*, Merriam-Webster.com, https://www.merriam-webster.com/dictionary/coccus (last visited May 21, 2017).

[2] "Pneumococcus is encapsulated with a chemically linked polysaccharide which confers serotype specificity. There are 90 known serotypes of pneumococci, and the capsule is the principle virulence determinant for pneumococci, as the capsule not only protects the inner surface of the bacteria from complement, but is itself poorly immunogenic." Ex. 1007, 2:10–14.

[3] The valency of a vaccine refers to the number of different serotypes of bacteria to which the vaccine induces immune response (*e.g*. a heptavalent vaccine protects against seven different bacterial strains).

D. Illustrative Claims

Claim 1, the sole independent claim of the '559 patent, is illustrative of the challenged claims and recites:

> 1. An immunogenic composition comprising a *Streptococcus pneumoniae* serotype 22F glycoconjugate, wherein the glycoconjugate has a molecular weight of between 1000 kDa and 12,500 kDa and comprises an isolated capsular polysaccharide from *S. pneumoniae* serotype 22F and a carrier protein, and wherein a ratio (w/w) of the polysaccharide to the carrier protein is between 0.4 and 2.

Ex. 1001, 141:28–34. Each of the remaining claims 2–45 depends directly or indirectly from claim 1.

E. The Asserted Grounds of Unpatentability

Petitioner contends that the challenged claims are unpatentable based on the following grounds. Pet. 4, 15, 17.

Reference	Basis	Claims Challenged
Pfizer-302[4]	§ 102(a)	1–18, 20, 22–27, 29–32, 35–45
Pfizer-302, GSK-711,[5] Merck-086,[6] GSK-531[7]	§ 103	3–9, 19, 21, 28, 33, 34

[4] Gu et al., WO 2014/027302 A1, published Feb. 20, 2014 ("Pfizer-302," Ex. 1009).
[5] Biemans et al., WO 2007/071711 A2, published June 28, 2007 ("GSK-711," Ex. 1007).
[6] Caulfield et al., US 2011/0195086 A1, published Aug. 11, 2011 ("Merck-086," Ex. 1008).
[7] Biemans et al., WO 2011/110531 A2, published Sept. 15, 2011 ("GSK-531," Ex. 1014).

Pfizer-099[8]	§ 102(a)	1, 3–14, 16–18, 20–32, 35–37, 39, 41, 42, 45
Pfizer-099, GSK-711, Merck-086, GSK-531, Lees-2008,[9] PVP 2013,[10] Pfizer-605,[11] Hsieh 2000[12]	§ 103	2, 3–9, 15, 19, 33, 34, 38, 40, 43, 44

Petitioner relies on the Declaration of Andrew Lees, Ph.D. Ex. 1006.

I. ANALYSIS

A. Priority and AIA 35 U.S.C. § 102(a)(1)

Petitioner asserts the '559 patent is not entitled to the priority date of its provisional application, US 61/929,547, because the provisional does not describe a polysaccharide to carrier protein ratio range "between 0.4 and 2," fails to describe sufficient species to support the molecular weight range between 1000 and 12,500 kDa, and only exemplifies a single CRM_{197} carrier protein that "cannot represent the entire genus of any carrier proteins." Pet. 15, 21, 23, 24, 26. Petitioner therefore asserts that Pfizer-099 and Pfizer-302

[8] Han et al., WO 2014/097099 A2, published June 26, 2014 ("Pfizer-099," Ex. 1010).
[9] Lees et al., "Chapter 11. Conjugation Chemistry," In: Pneumococcal Vaccines: The Impact of Conjugate Vaccine (Ed. George R. Siber et al.); pp. 163–174 (2008) ("Lees-2008," Ex. 1011).
[10] "Pneumococcal Vaccine Polyvalent" revision to Japan's "Minimum Requirements for Biological Products" published on the website of Japan's National Institute of Infectious Diseases (as of March 2, 2013) ("PVP 2013," Ex. 1012).
[11] Prasad, A.K., US 7,955,605 B2, issued June 7, 2011 ("Pfizer-605," Ex. 1013).
[12] Hsieh, *Characterization of Saccharide-CRM_{197} Conjugate Vaccines*, In: *Physico-Chemical Procedures for the Characterization of Vaccines* (Eds. Brown F., Corbel M., and Griffths E.); Vol. 103, pp. 93–104; Basel; Karger, 2000 ("Hsieh 2000," Ex. 1015).

are prior art to the '559 patent under AIA 35 U.S.C. § 102(a)(1) or 35 U.S.C. § 102(a)(2). Pet. 15, 17.

Patent Owner asserts the "'559 patent is entitled to its priority date." Prelim. Resp. 16. Patent Owner relies on *Wertheim* for the proposition that "a claimed range may be supported by the combination of a generic range and specific embodiments disclosed in a patent application." Prelim. Resp. 29 (*citing In re Wertheim*, 541 F.2d 257, 265 (CCPA 1976)). Patent Owner asserts both the '547 provisional and the '559 patent show a Table 16 with glycoconjugate batches with polysaccharide to protein ratios of 0.4 and 2, providing descriptive support for the claimed range of a "polysaccharide to the carrier protein [that] is between 0.4 and 2." Prelim. Resp. 29–30, 33–34. Patent Owner notes Table 16 shows "numerous ratios falling within the claimed range (0.75, 0.87, 0.8, 0.8, 1.9, 0.8, 0.65 and 1.0)." Prelim. Resp. 33−34 (citing Ex. 1002, 16).

Patent Owner asserts, regarding the molecular weight range of 1000 kDa to 12,500 kDa in claim 1 of the '559 patent, that the "'547 application teaches the generation and characterization of sufficient representative species encompassed by the '559 patent claims." Prelim. Resp. 35–36. Patent Owner asserts, regarding the breadth of carrier proteins encompassed by claim 1 of the '559 patent, that "the '547 application discloses a number of possible specific carrier proteins" and that "[b]y listing these carrier proteins, the '547 application adequately describes numerous carrier proteins, not just one as contested by Sanofi, for potential inclusion in the claimed compositions." Prelim. Resp. 39.

Benefit of priority depends upon whether there is descriptive support for claim 1 of the '559 patent in the '547 provisional. "A reference patent is only entitled to claim the benefit of the filing date of its provisional application if the disclosure of the provisional application provides support for the claims in the reference patent in compliance with § 112, ¶ 1." *Dynamic Drinkware, LLC v. National Graphics, Inc.*, 800 F.3d 1375, 1381 (Fed. Cir. 2015).

We are persuaded that the '547 provisional supports the immunogenic composition recitation in claim 1 because it teaches the "present invention relates to new immunogenic compositions . . . [that] typically comprise conjugated capsular saccharide antigens . . . derived from serotypes of *S. pneumoniae*." Ex. 1002, 17. We are also persuaded that the '547 provisional supports the serotype 22F molecular weight recitation in claim 1 because it teaches embodiments where "the serotype 22F glycoconjugate has a molecular weight of . . . between 1,000 kDa and 12,500 kDa." Ex. 1002, 38. The '547 provisional also provides ten examples of conjugated serotype 22F molecular weights ranging from 1419 kDa to 10,450 kDa, further demonstrating examples within the claimed range. *See* Ex. 1002, 116.

We are persuaded that the '547 provisional supports the recitation in claim 1 of the '559 patent requiring a "ratio (w/w) of the polysaccharide to the carrier protein [that] is between 0.4 and 2." Ex. 1001, 89. The '547 provisional teaches "the ratio of serotype 22F polysaccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3 (e.g. about 0.5 . . . [)] . . . . In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 2." Ex. 1002, 39. In addition to these general range teachings, the '547 provisional provides ten specific examples of

saccharide/protein ratios within the range of 0.4 and 2, including batch 6 with a saccharide/protein ratio of 0.4 and batch 3 with a saccharide/protein ratio of 2. *See* Ex. 1002, 116.

We are not persuaded by Petitioner's argument that the disclosure of a range and the exemplary batches "is not a description for the range of 'between 0.4 and 2.0' in full, clear, concise, and exact terms, even if the ratio of Batch 6 in Table 16 is combined with the range of 'between 0.5 and 2' disclosed in the specification." Pet. 22.

In *Wertheim*, the predecessor to our reviewing court explains that "in light of the description of the invention as employing solids contents within the range of 25-60% along with specific embodiments of 36% and 50%, we are of the opinion that, as a factual matter, persons skilled in the art would consider processes employing a 35-60% solids content range to be part of appellants' invention." *In re Wertheim*, 541 F.2d 257, 265 (CCPA 1976). Similarly, the disclosure in the '547 provisional of both a range for the polysaccharide/protein ratio of between "0.5 and 2" along with a ratio "about 0.5" and specific embodiments of 0.4 and 2 reasonably provide descriptive support for a polysaccharide/protein ratio range between 0.4 and 2 as recited in claim 1 of the '559 patent. *See* Ex. 1002, 39, 116.

The fact pattern in this case is different than the facts in the cases relied upon by Petitioner. *See* Pet. 22. In *Ahlbrecht*, there was "nothing in the original specification to indicate that any other esters (i.e., those where m is 2 or greater than 12) may be made by the methods disclosed." *In re Ahlbrecht*, 435 F.2d 908, 911 (CCPA 1971). In the instant case, both the "about 0.5" language, which suggests the adjacent range value of 0.4, and

the specific exemplification of a polysaccharide/protein ratio of 0.4 demonstrate that this embodiment was described and enabled by the '547 provisional. *See* Ex. 1002, 39, 116. In *Blaser*, there was no description of the value at issue in the Specification of the priority document, unlike the current situation where there is a specific example of a 0.4 ratio of polysaccharide/protein. *See In re Blaser*, 556 F.2d 534, 538 (CCPA 1977); Ex. 1002, 116. Similarly, in *Lukach*, the court explained that a "single example [of an Mw/Mn ratio of 2.6] does not alone provide support for the recited range from 2.0 to 3.0, and nothing has been brought to our attention to show that any other language in the grandparent application, taken together with the knowledge of persons skilled in the art, points to the recited range." *In re Lukach*, 442 F.2d 967, 969 (CCPA 1971). However, in the '547 provisional, in addition to the disclosure of a range for the polysaccharide/protein of between 0.5 and 2, and a disclosure of a value of about 0.5, the '547 provisional has ten examples of 22F polysaccharide/protein ratios that range from 0.4 to 2. *See* Ex. 1002, 39, 116. These disclosures in the '547 provisional reasonably satisfy the written description requirement and provide descriptive support for the range in claim 1 of the '559 patent of a "ratio (w/w) of the polysaccharide to the carrier protein [that] is between 0.4 and 2." Ex. 1001, 89.

We are not persuaded by Petitioner's argument that "out of the whole range span of the claimed genus (from 0.4 to 2, which is 1.6), there is no description of more than half of the claimed range (from 1 to 1.9, which is 56% of the whole genus (i.e., 0.9/1.6))." Pet. 23. Nor are we persuaded by Petitioner's argument that the "vast majority of variations with respect to

this combination are not represented by the examples disclosed in the provisional application." Pet. 25.

"An adequate written description must contain enough information about the actual makeup of the claimed products—'a precise definition, such as by structure, formula, chemical name, physical properties, or other properties, of species falling within the genus sufficient to distinguish the genus from other materials.'" *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1378 (Fed. Cir. 2017) (*citing Ariad Pharm., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1350 (Fed. Cir. 2010) (*en banc*)). Both the '547 provisional and claim 1 of the '559 patent provide a structural formula composed of a ratio between the polysaccharide and the carrier protein, with the '547 provisional expressly disclosing a range between 0.5 and 2, a specific value of about 0.5, and ten examples of serotype 22F polysaccharide/protein ratios including ratios from 0.4 to 2. *See* Ex. 1002, 39, 116. The '547 provisional further provides chemical names and structural information for both the carrier proteins and the serotype 22F polysaccharides. See Ex. 1002, 20–21, 29. Thus, the '547 provisional provides enough information about the actual makeup of the range of serotype 22F glycoconjugates to distinguish the genus from materials not falling within the scope of claim 1 of the '559 patent and providing descriptive support for that claim.

We are not persuaded by Petitioner's argument that:

> The genus of carrier proteins is vast. Lees ¶135. The provisional application itself discloses at least 55 different possible carrier proteins that can be used to conjugate to each individual serotype. Ex. 1002, 20:12–21:2; Lees ¶135. These carrier proteins share no common structural features and are derived from completely different sources. Lees ¶135.

> Therefore, a single example of CRM_{197} cannot represent the entire genus of any carrier proteins. Lees ¶ 135.

Pet. 26.

We are persuaded that the '547 provisional provides support for the genus of carrier proteins because it teaches a large number of different carrier proteins, while recognizing that "[c]arrier proteins should be amenable to standard conjugation procedures." Ex. 1002, 20–21. "[T]he determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter." *Capon v. Eshhar*, 418 F.3d 1349, 1359 (Fed. Cir. 2005). The '547 provisional cites literature sources for each of the many different cited carrier proteins, including both patent and non-patent references, demonstrating that selection of carrier proteins was a predictable choice made based on the extensive knowledge in the field of vaccine production. *See* Ex. 1002, 20–21.

Dr. Lees provides no evidentiary support for the position that a "single example of CRM_{197} cannot adequately represent the entire genus of any carrier protein." Ex. 1006 ¶ 135. Indeed, Dr. Lees cites other prior art that demonstrates the predictability of conjugation of pneumococcal saccharides to other carrier proteins specifically "GSK-711 also discloses that the saccharides present in the immunogenic composition (such as 22F) may be conjugated to a carrier protein independently selected from CRM197, diphtheria toxoid (DT), tetanus toxoid (TT), pneumococcal pneumolysis (Ply), polyhistidine triad proteins (PhtX proteins such as PhtD proteins), or

Haemophilus influenzae protein D (PD)." Ex. 1006 ¶ 90 (citing Ex. 1007, 9, 11).

> We are not persuaded by Petitioner's argument that:
>
> > Pfizer itself has admitted that other carrier proteins are not always substitutable and that CRM_{197} is unique because it unexpectedly solved the immunogenicity problem in a 13-valent PCV composition while other carrier proteins cannot. *E.g.*, *Merck Sharp & Dohme Corp. v. Wyeth LLC*, 2017 WL 3160412, IPR2017-01215 paper 8 at 28–36 (PTAB July 25, 2017).

Pet. 27. In Paper 8 of IPR 2017-01215, Patent Owner states that the prior art "directed a POSA to utilize multiple carriers in a conjugate-based vaccine" due to a concern over carrier-induced epitopic suppression (CIES). *Merck Sharp & Dohme Corp. v. Wyeth LLC*, IPR2017-01215 paper 8 at 30 (PTAB July 25, 2017). Moreover, Patent Owner noted a prior art "mixed carrier vaccine comprising use of protein D, tetanus toxoid, and diphtheria toxoid." *Id*. at 31. Thus, this evidence tends to support, rather than rebut, the expectation that a general description of carrier proteins for use in vaccines provides descriptive support because these carrier proteins are routinely and predictably used for vaccine formulations. *Id*. at 30–31. That unexpected improvements may be identified for specific carrier proteins does not undermine descriptive support based on the '547 provisional's recitation of the chemical names and prior art related to a large number of known carrier proteins. Ex. 1002, 20–21. Therefore, the evidence currently of record does not provide a reasonable likelihood that there is any unpredictability or lack of knowledge in the mature field of conjugating carrier proteins to saccharides to form polysaccharide conjugate vaccines.

Because we find that the '559 patent receives benefit of priority to the '547 provisional with a priority date of January 21, 2014, neither Pfizer-302 nor Pfizer-099 are prior art under AIA 35 U.S.C. §102(a)(1) and cannot serve as the basis for either anticipation or obviousness under that section.

B. AIA 35 U.S.C. § 102(a)(2) [13]

Petitioner asserts: "In the event the Board determines that the '559 patent is entitled to the priority date, Pfizer-302 is prior art to the '559 patent under AIA 35 U.S.C. § 102(a)(2)" and "Pfizer-099 is prior art to the '559 patent under AIA 35 U.S.C. § 102(a)(2)." Pet. 15, 17.

Patent Owner asserts "Pfizer-302 and Pfizer-099 [] are not prior art pursuant to AIA 35 U.S.C. § 102(b)(2)(C) because the references and the claimed invention were, as of the effective filing date of the claimed invention, commonly owned by and subject to an obligation to assignment to Pfizer." Prelim. Resp. 24. Patent Owner:

> submits assignment documents[] and declarations[] from PCT request forms[] confirming that all of the inventors listed on Pfizer-302, Pfizer-099, the '547 application and the '559 patent assigned, and had an obligation to assign, these patent application filings to Pfizer. EX2005 at 3−12; EX2006 at 3−16; EX2007 at 5−7; EX2008 at 3−5; EX2009 at 5−7; EX2010 at 5−7. Further, Sanofi has not provided any evidence that raises a material doubt as to Pfizer's claim of common ownership.

Prelim. Resp. 25.

[13] We note that MPEP § 2152.04 states "the Office is treating the term 'disclosure' as a generic expression intended to encompass . . . WIPO published application[s]." We note that both Pfizer-302 and Pfizer-099 are WIPO published applications and therefore included as prior art under AIA 35 U.S.C. § 102(a)(2).

"A disclosure shall not be prior art to a claimed invention under subsection (a)(2) if— . . . (C) the subject matter disclosed and the claimed invention, not later than the effective filing date of the claimed invention, were owned by the same person or subject to an obligation of assignment to the same person." AIA 35 U.S.C. § 102(b)(2)(C).

We are persuaded that Pfizer-302 and Pfizer-099 are not prior art to the '559 patent under AIA 35 U.S.C. § 102(a)(2) because they fall within the exception to that provision articulated in AIA 35 U.S.C. § 102(b)(2)(C). In particular, we find that Patent Owner provided evidence that these publications were subject to assignment to Patent Owner as of the effective filing date of the '559 patent.[14] The assignments were recorded in the '547 provisional by February 11, 2014, prior to the February 20, 2014 publication of Pfizer-302 and the June 26, 2014 publication of Pfizer-099. Ex. 2005, 1–2; Ex. 1009, 1; Ex. 1010, 1. Both Pfizer-302 and Pfizer-099 were also subject to assignment to Patent Owner. *See* Ex. 2009, 1–9; Ex. 2010 1–9. Additionally, Patent Owner submits assignment to Patent Owner of the U.S. 14/597,488 application to leading to the '559 patent as well. Ex. 2006, 1–2.

Therefore, Pfizer-302 and Pfizer-099 are not prior art to the '559 patent under AIA 35 U.S.C. § 102(a)(2) because they are excluded under AIA 35 U.S.C. § 102(b)(2)(C) as subject to an obligation of assignment to the same person, here Patent Owner.

[14] We note that MPEP § 2154.02(c) states: "If the provisions of AIA 35 U.S.C. 102(b)(2)(C) are met, a U.S. patent document that might otherwise qualify as prior art under AIA 35 U.S.C. 102(a)(2) is not available as prior art under either AIA 35 U.S.C. 102 or 103."

III. CONCLUSION

After reviewing the information presented in the Petition and the Preliminary Response, as well as the evidence of record thus far, we determine that Petitioner has not established a reasonable likelihood that it will prevail in showing that claims 1–45 of the '559 patent are unpatentable.

IV. ORDER

Accordingly, it is

ORDERED that Pursuant to 35 U.S.C. § 314(a), the petition for *inter partes* review is hereby denied as to all challenged claims and no trial is instituted.

IPR2018-00188
Patent 9,492,559 B2

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EXHIBIT D

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

UNITED THERAPEUTICS CORPORATION,)
)
----------Plaintiff,)
) Case No.
vs.) 23-CV-975-RGA
)
LIQUIDIA TECHNOLOGIES, INC.,)
) Volume I
----------Defendant.)

TRANSCRIPT OF BENCH TRIAL

BENCH TRIAL had before the Honorable Richard G. Andrews, U.S.D.C.J., in Courtroom 6A on the 23rd of June, 2025.

APPEARANCES

MORRIS, NICHOLS, ARSHT & TUNNELL LLP
BY: MICHAEL FLYNN, ESQ.

-and-

GOODWIN PROCTER LLP
BY: WILLIAM JACKSON, ESQ.
KATIE CHENG, ESQ.
ERIC ROMEO, ESQ.
ERIC LEVI, ESQ.

-and-

MCDERMOTT WILL & EMERY
BY: DOUG CARSTEN, ESQ.
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Counsel for Plaintiff

(Appearances continued.)

SHAW KELLER LLP
BY: NATHAN HOESCHEN, ESQ.
KAREN KELLER, ESQ.

-and-

COOLEY LLP
BY: SANYA SUKDUANG, ESQ.
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JORDAN LANDERS, ESQ.
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ROBERT MINN, ESQ.
ANDREW LAU, ESQ.

Counsel for Defendant

THE COURT: Mr. Jackson, are you ready to begin?

MR. JACKSON: Yes, Your Honor.

THE COURT: And, Mr. Sukduang, are you ready to begin?

MR. SUKDUANG: Yes, Your Honor.

THE COURT: All right. Well, then let's begin.

MR. JACKSON: Your Honor, I'm going to -- for my opening statement, I have a set of demonstratives.

May I approach?

THE COURT: Sure.

MR. JACKSON: May it please the Court. Good morning, Your Honor. Thank you for having us.

This is -- as you know, this is the second Hatch-Waxman case between these parties before the Court. It's between UTC, which I represent, and Liquidia. This one is for a new indication which is different from the last case.

Let me reintroduce the parties. First UTC, that's my client. UTC is an innovator. They started focused on finding solutions for something called pulmonary hypertension, which is just hypertension of the pulmonary circuit, heart and lungs. Hence the logo.

They are now doing lots of research into many different areas, research in new therapies and new solutions for a variety of different maladies. Liquidia is staffed by

a number of former UTC executives who left us and went into Liquidia, including a number of their most senior executives.

In this case -- the case will involve their pulmonary hypertension. There are five types of pulmonary hypertension. The last case was pulmonary arterial hypertension, or PAH. That's group one. This case -- this is what the world's experts have defined pulmonary hypertension as being.

This case is about what would fall within group three, which is pulmonary hypertension due to lung disease, which is -- includes interstitial lung disease and COPD.

As of April 2020, which is the priority date of the patent, UTC and other companies have a number of drugs that were approved and a number of therapies that were approved for PAH, for group one. There were no drugs approved for PH-ILD, none.

But that's not for lack of trying. Lots of therapies have been tried for PH-ILD. This slide identifies what I have referred to as the seven deadly studies. These are seven studies that were attempted for Group 1 medications, that's PAH medications, for use in Group 3 populations.

All of these studies have been -- involved randomized clinical trials for a drug used for PAH and

documents presented by UTC, and Dr. Rothblatt's public statements confirm not only the sale of Tyvaso, but the specific use of the claimed method treating PH-ILD and expressed in exercise capacity, but it establishes the motivation and expectation based upon those posters and publications to use treprostinil in an inhaled form according to dosing in the '327 label -- patent to treat PH-ILD patients and improve their exercise capacity.

Your Honor, they asserted Claims 1, 5, 6, 9, 14, and 17. The Court has construed terms on that. The experts in this case have applied those constructions to their analysis both on noninfringement and invalidity.

And you'll hear from Dr. Channick regarding noninfringement of Claims 5, 6, 9, and 17, that those claims are not directly infringed by any doctor, and that the Yutrepia label and Liquidia does not induce infringement.

Counsel brought up the idea of this -- a belief on invalidity. Your Honor, that's not an argument that we're making. The label does not direct, instruct, encourage to meet the outcomes that are claimed and required to be measured by these claims.

You'll hear from Dr. Channick regarding the obviousness based on Faria '793 and Saggar. You'll hear from Dr. Channick regarding anticipation. And under controlling federal circuit precedent, the issue is not

whether virtually all patients receive an outcome. The issue is whether the outcome will necessarily inevitably happen in this patient population.

The Court construed Claim 1 to be one patient. The INCREASE study, which forms the basis of the '327 claims, does not cover all patients. Therefore, the claim is inherently anticipated.

You'll hear from Dr. Channick regarding the lack of written description. For some reason counsel indicates that Claim 9 doesn't require absolute and percent predicted FEC. That is wrong as a matter of law.

Claim 10, which they originally asserted but now have dropped, specifically depends from Claim 9 and is specific to absolute FEC. Claim 9 includes, encompasses, however you want to say it, for absolute and percent predicted.

And finally, you'll hear from Dr. Hill regarding the prior sale of Tyvaso, specifically for the claimed method prior to 2020.

You've met Dr. Hill in the past. You'll meet Dr. Channick, who's at UCLA. Dr. Nathan was part of the Sixth World Symposium to set up guidelines on PDH-ILD. Those guidelines have now been revamped and replaced by guidelines that Dr. Channick helped develop because he was the co of the task force lead for the Seventh World

Symposium on PH-ILD and PH with chronic lung diseases.

And that's the language that is now on the label, pulmonary hypertension associated with interstitial lung disease.

But we thought that UTC was going to present commercial success through their expert Dr. Selck. They now acknowledge that they're not. We don't expect to hear from Dr. Selck. If he does appear, our expert Dr. Kidd will respond to that.

We heard that you'll hear from Dr. Thisted regarding the lack of value of small studies. Dr. Ogenstad will address that. And specifically, you'll see that despite Dr. Thisted's opinions, UTC, throughout their papers, both public and private, rely on the very studies that form the obviousness of this invention to support the studies and increase.

Your Honor, Yutrepia is on the market. Liquidia is getting reports from patients that -- how their lives have now changed to be able to use Yutrepia. This has been a long haul 20 years for Liquidia. You've been a part of that, to some extent, and we appreciate that, and we appreciate your time. Thank you.

THE COURT: I take it the judge in North Carolina denied either the TRO, the preliminary injunction, or both?

MR. SUKDUANG: Correct. The judge determined --

THE COURT: I don't need to hear any more.

MR. SUKDUANG: Yes, you're absolutely right.

THE COURT: And do I also take it, as Mr. Jackson said, that you stipulated to infringement of Claim 1 and 14 both direct and indirect?

MR. SUKDUANG: Correct, Your Honor.

THE COURT: All right. Thank you.

MR. SUKDUANG: Thank you.

MS. KELLER: Your Honor, Karen Keller from Shaw Keller on behalf of Liquidia. One quick request by Liquidia, Your Honor. We would request under Federal Rule of Evidence 615 that the fact witnesses that are in the gallery be sequestered until they testify.

THE COURT: All right. That will be granted.

MS. CHENG: Good morning, Your Honor. Katie Cheng from Goodwin Procter on behalf of United Therapeutics.

THE COURT: All right. Good morning, Ms. Cheng.

MS. CHENG: United Therapeutics would like to call Dr. Noah Byrd.

THE COURT: All right.

MS. CHENG: Your Honor, I also have a very small binder.

May I approach?

THE COURTROOM DEPUTY: Please state your name

and spell it for the record.

THE WITNESS: Noah Byrd, N-O-A-H, B-Y-R-D.

NOAH BYRD,

called as a witness on behalf of the Plaintiff, was sworn, and testified as follows:

DIRECT EXAMINATION

BY MS. CHENG:

Q. Good morning, Dr. Byrd.

A. Good morning.

Q. Could you please introduce yourself to the Court.

A. Sure. My name is Noah Byrd. I am the vice president of global regulatory affairs of United Therapeutics.

Q. Is United Therapeutics referred to by a shorthand name?

A. Yes, also by UT or UTC.

Q. What are your responsibilities as vice president of global regulatory affairs at UTC?

A. At a high level, I have regulatory responsibility and regulatory strategic supports for our commercial portfolio as well as for all of our products in development.

Q. Do your responsibilities also include overseeing regulatory submissions to agencies like the FDA?

A. Yes.

Q. How long have you been working at UTC?

A. A little bit over 15 years.

Q. During your time at UTC, have you developed a high-level understanding of what UTC does?

A. Yes, at a high level.

Q. At a high level, what does UTC do?

A. We develop drugs for patients with rare and chronic diseases.

Q. Are there any particular diseases on which UTC primarily focuses?

A. I would say our primary focus is on PAH and PAH associated diseases.

Q. What does "PAH" stand for?

A. Pulmonary arterial hypertension.

Q. What does "PH" stand for?

A. Pulmonary hypertension.

Q. You've been with UTC for about 15 years.

In that time, have you developed a high-level understanding of the history of the company?

A. In general terms, yes.

Q. Approximately when was UTC founded?

A. 1996, 1997.

Q. Why was UTC founded?

A. My understanding is that the company was founded essentially in response to a parent learning that their

child had been diagnosed with a rare life-threatening disease. The founder is Dr. Martine Rothblatt and the child is Jenesis Rothblatt.

At the time of diagnosis, there were no satisfactory treatments for PAH and so Dr. Rothblatt made it her mission to develop one. And that's what led to our first drug being approved in 2002, Remodulin.

Q. And has UTC continued to further develop products after it brought Remodulin to market?

A. Yes, we have.

Q. Are you familiar with UTC's ongoing research?

A. Yes, I am.

Q. What kinds of research has UTC been engaged in during the time you've been at the company?

A. I started the company in 2010. Prior to that, we had subcutaneous and IV development. Just before joining, we got approval for inhaled treprostinil drug called Tyvaso. Once I joined, I continued to work on that product through life cycle modifications. We developed a drug for a rare pediatric cancer called neuroblastoma. We developed an oral formulation for treprostinil called Orenitram. We expanded additional indications PH-ILD with recent approval of the other formulation of treprostinil called Tyvaso DPI. We continue to expand in areas beyond xenotransplantation, things of that nature.

Q. You just mentioned several products that UTC has developed. Have you prepared a slide today showing the products that UTC offers?

A. I have.

Q. I'd like to pull that up and go to slide 2. What does this slide show?

A. This slide shows the drugs that we have U.S. FDA approval for and that are for sale for commercial distribution in the U.S.

Q. Which of these products listed on the slide are based on treprostinil?

A. The Remodulin, Tyvaso, Orenitram and Tyvaso DPI.

Q. You also mentioned these products have been approved in the U.S. by the FDA. What does approval by the FDA allow UTC to do with these products generally?

A. It allows us to sell them for patients.

Q. As part of your role at UTC, have you developed any familiarity with the indications for which UTC's treprostinil-based products are approved?

A. At a high level.

Q. What indications is Tyvaso, the nebulized product, approved for?

A. PAH, treatment of PAH.

Q. Any other indications for Tyvaso nebulized?

A. Also treatment of PH-ILD.

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Q. What does PH-ILD stand for?
A. Pulmonary hypertension associated with interstitial lung disease.
Q. When did you receive approval for PAH for Tyvaso?
A. I believe that was in 2009.
Q. When did UTC receive approval for PH-ILD for Tyvaso?
A. That was in March 2021.
Q. Why did Tyvaso receive approval for PH-ILD some years after its approval for PAH?
A. That was a different clinical development program.
Q. Why did UTC need a different clinical development program for the PH-ILD indication?
A. Because that's a different patient population than patients with PAH.
Q. Do you know whether UTC had to conduct additional studies to obtain approval for Tyvaso in PH-ILD?
A. Yes. That was a clinical study known as INCREASE.
Q. Switching to Tyvaso DPI, when did UTC receive approval for PAH for Tyvaso DPI?
A. I believe that was in May 2022.
Q. When did UTC receive approval for PH-ILD for Tyvaso DPI?
A. That was at the same time in May 2022.
Q. Why did UTC get approval for Tyvaso DPI and later for the Tyvaso the nebulized product?

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A. That was an entirely new product.
Q. For the new product, did UTC need a different clinical development program?
A. We did.
Q. Do you have any understanding of what studies were involved in the clinical development program for the DPI product?
A. At a high level.
Q. What were those studies?
A. There were two pharmacokinetics studies conducted in healthy normal volunteers and there was one study called Breeze, which is a switch study in patients with PAH.
Q. Before UTC got approval for PH-ILD for Tyvaso nebulized, could UTC market Tyvaso nebulized for the PH-ILD indication?
A. No.
Q. Before UTC got approval for Tyvaso DPI, could UTC market the dry powder product for PH-ILD indication?
A. No.
Q. Why could UTC not market the two Tyvaso products for PH-ILD prior to their receiving approval for that indication?
A. Because you need approval by the FDA before you can sell its products or enter them into commercial distribution.

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Q. Have you ever signed off on UTC engaging in marketing of Tyvaso or Tyvaso DPI for indications that it did not have approval for?
A. No.
Q. In your experience at UTC, how seriously, if at all, is marketing approval taken?
A. Very serious.
Q. To your knowledge, did UTC market Tyvaso or Tyvaso DPI for PH-ILD before it had approval?
A. No.
Q. Switching topics, do you have any familiarity with UTC's patent portfolio?
A. At a high level.
Q. How did you come to have a high level understanding of UTC's patents?
A. I'm aware of the fact they're listed in a reference manual called the *Orange Book*, and as part of our regulatory function, we also assist the patents to marketing applications and supplements.
Q. You mentioned the *Orange Book*. Can you just provide a high level understanding -- high level overview and your understanding of what the *Orange Book* is?
A. Sure. It's essentially a record of FDA-approved drugs and their associated patents.
Q. Are you aware of the patents that UTC has listed in

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the *Orange Book* for Tyvaso?
A. At a high level, yes.
Q. Have you prepared a slide showing the patents that UTC listed in the *Orange Book* for Tyvaso?
A. I have.
Q. I'd like to go to slide 3, please. What does this slide show?
A. It shows the entry for treprostinil Tyvaso and its eight patents and their expiration dates.
Q. Can you identify the patents that UTC has listed in the *Orange Book* for Tyvaso?
A. I can. Would you like me to read them?
Q. Please.
A. 10,376,525; 10,716,793; 1,172,387; 11,826,327; 9,339,507; 9,358,240; 9,593,066; 9,604,901.
Q. Does UTC currently own all the patents listed on the slide ?
A. Yes.
Q. Has UTC always owned the issued patents listed on the slide from the date each one was first filed?
A. Yes.
Q. I see there's an answer at the bottom of this slide regarding the '793 patent. Can you explain briefly what that's in reference to?
A. Yes. The '793 patent was subsequently delisted from

the *Orange Book*.

Q. And approximately when did that occur, if you know?

A. Generally within the last year.

Q. I'd like to now have you turn in your binder to the document marked as JTX 0001. Do you recognize this document?

A. I do.

Q. What is this document?

A. This is Patent '327.

MS. CHENG: If we could admit JTX 0001. I understand there are no objections.

UNIDENTIFIED SPEAKER: No objection.

THE COURT: All right. Admitted without objection.

(Thereupon, Joint Exhibit JTX 0001 was admitted.)

BY MS. CHENG:

Q. Is the '327 patent in JTX 0001 the same patent that was listed on the *Orange Book* slide we just looked at?

A. It is.

Q. Is the '327 patent the patent that is being challenged in this case?

A. Yes. That's my understanding.

Q. You mentioned at the beginning that UTC is currently engaged in additional research and development?

A. Yes.

Q. Have you prepared a slide with examples of UTC's ongoing research?

A. I have.

Q. Go to slide 4, please.

Can you provide a high level overview of the kind of research and development that UTC is currently engaged in?

A. Sure. This is two examples. We have the Teton program, which is an exploration of Tyvaso in new patient populations comprised of IPF, idiopathic pulmonary fibrosis, and PPF, progressive pulmonary fibrosis. We have three basic studies in the Teton program.

And we also have -- in an effort to provide a -- supply limitless transplantable organs, we have a number of shots on goal. One of them is a generation of genetically edited pigs as a source for those organs. And we currently have a clinical study underway for tanginoted and xenokidneys for patients with end-stage renal disease.

Q. What drives UTC to continue investing in research and development in these potential products and therapies?

A. I think it comes from the top down from. Our founder and CEO Martine Rothblatt, she's a visionary and a frontier blazer and I think that it's culture of the company we constantly strive to innovate and provide our patients with

topnotch therapeutic options.

MS. CHENG: Thank you, Dr. Byrd. No further questions.

THE COURT: All right. Thank you, Ms. Cheng.

CROSS-EXAMINATION

BY MR. MORTON:

Q. Good morning.

A. Good morning.

Q. You testified and you're testifying with Ms. Cheng that you're responsible for UTC's regulatory submissions to the FDA?

A. Yes, in general terms.

Q. And those regulatory submissions to the FDA, those include investigative brochures?

A. Yes.

Q. Do you recall being deposed by my colleague in this matter?

A. Colleague who?

Q. What -- one of my colleagues in this matter?

A. Yes.

Q. In your binder, there's a document marked DTX 387. Please turn to that.

Do you recall being shown this document in your deposition, Dr. Byrd?

A. Yes, I believe so.

Q. And in that, in your deposition, you testified that it was an investigative brochure for treprostinil inhalation dated August 26, 2016; correct?

A. Yes, that's what it appears to be.

Q. You testified this document would have been submitted to the FDA; correct?

A. Yes, I believe so.

Q. And you testified -- turn to page 10 of DTX 387.

You testified that the 2016 investigative brochure summarize the teaching of several publications including Saggar 2009, Saggar 2014 and Agarwal 2015 as shown on pages 10 and 11 of DTX 387?

A. Okay.

Q. You did testify to that; yes?

A. I believe so, yes.

Q. And if you turn to page 131 of DTX 387. There's a reference to the citation to the Agarwal 2015 paper. Do you see that?

A. Yes.

Q. And UTC provided that citation to the FDA?

A. I believe so.

Q. And if you turn to the next page, 132, of DTX 387. UTC provided the citation to Saggar 2014. That's the third from the top?

A. I believe so. I think I stated that at the time, I

EXHIBIT E

IN THE UNITED STATES DISTRICT COURT

IN AND FOR THE DISTRICT OF DELAWARE

UNITED THERAPEUTICS CORPORATION,)
)
----------Plaintiff,)
) Case No.
vs.) 23-CV-975-RGA
)
LIQUIDIA TECHNOLOGIES, INC.,)
) Volume II
----------Defendant.)

TRANSCRIPT OF BENCH TRIAL

BENCH TRIAL had before the Honorable Richard G. Andrews, U.S.D.C.J., in Courtroom 6A on the 24th of June, 2025.

APPEARANCES

MORRIS, NICHOLS, ARSHT & TUNNELL LLP
BY: MICHAEL FLYNN, ESQ.

-and-

GOODWIN PROCTER LLP
BY: WILLIAM JACKSON, ESQ.
KATIE CHENG, ESQ.
ERIC ROMEO, ESQ.
ERIC LEVI, ESQ.

-and-

MCDERMOTT WILL & EMERY
BY: DOUG CARSTEN, ESQ.
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(Appearances continued.)

SHAW KELLER LLP
BY: NATHAN HOESCHEN, ESQ.
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BY: SANYA SUKDUANG, ESQ.
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JORDAN LANDERS, ESQ.
RACHEL PRESTON, ESQ.
ROBERT MINN, ESQ.
ANDREW LAU, ESQ.

Counsel for Defendant

THE COURT: So is there some problem I need to address?

MR. CARSTEN: Yes, Your Honor. So as Your Honor will likely recall, Your Honor limited us to six claims and Liquidia to four defenses, and then issued a subsequent order that said for purposes of counting obviousness, one counting is a defense.

THE COURT: I did.

MR. CARSTEN: They submitted their election of defenses and then on the same day, Your Honor reversed the magistrate judge's order and struck two of those defenses.

THE COURT: Yes.

MR. CARSTEN: They came back with an amended reduced set of defenses, prior sale, anticipation by the 2017 increase protocol, obviousness in view of a combination of Faria-Urbina, the '793 patent, and Saggar 2014, and the written description.

THE COURT: Yes.

MR. CARSTEN: And last night we received a demonstrative to be used with Dr. Channick in which Dr. Channick is rendering an opinion that Faria-Urbina 2018 and the '793 patent render Claim 1 obvious. That's not the same combination that they identified in their election of defenses. Now --

THE COURT: And I'm sorry, you say the slide is

Faria-Urbina and what?

MR. CARSTEN: Yes, Your Honor, it's Faria-Urbina and the '793 patent. It is not the Saggar reference.

THE COURT: So the same combination minus one.

MR. CARSTEN: Yes. So sort of a lesser included offense, if you have it, that they're arguing.

THE COURT: So what's the problem? Because presumably them getting -- is that, in fact, what you want to do?

MR. KNAUSS: Yes, Your Honor. Good morning. David Knauss.

THE COURT: Okay. Well, so just hold your through there.

MR. KNAUSS: Sure.

THE COURT: What's the problem with having an obvious combination based on two of the three rather than all three of the three?

MR. CARSTEN: Well, Your Honor has specifically indicated that a single combination is the defense and --

THE COURT: I understand what I indicated, but if they said, okay, it's the three and then they just don't mention the third, they either prove it or they don't prove it. I mean, something where you knock out one of the three that you're relying on, I don't see how there's the slightest bit of prejudice to you.

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A. It is further evidence.
MR. DAVIES: Can we please go to DTX 363.
BY MR. DAVIES:
Q. And I believe we've seen DTX 363 already as well; correct, Dr. Channick?
A. Yes.
Q. And what is DTX 363?
A. This is *The New England Journal* publication of the INCREASE study.
MR. DAVIES: Can we please go to page 2.
BY MR. DAVIES:
Q. What are the pilot -- well, Dr. Channick, can you read that statement that's highlighted there.
A. "Data from previously completed pilot studies suggest that inhaled treprostinil can improve hemodynamics and functional capacity in patients with Group 3 pulmonary hypertension."
Q. And then that cites a number of references.
Do you see that?
A. Yes.
Q. What does functional capacity mean in this context?
A. It means a lot of things. It means, as I've stated, how a patient functions, their exercise, their daily living, functional capacity.
Q. Can it refer to their exercise ability?

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A. That's part of the function, yes.
MR. DAVIES: Can we take a look at the Footnotes 9 and 10 that are cited for that statement.
BY MR. DAVIES:
Q. And what are the documents that are referenced in Footnotes 9 and 10?
A. These are Faria-Urbina and Agarwal.
Q. And how, if at all, does this inform your opinion regarding a POSA's reasonable expectation of success with respect to Claim 1?
A. Again, it supports it.
Q. After reviewing the publications and the other documents and considering your own experience and those of other POSAs, what is your opinion regarding whether a POSA would have a reasonable expectation of success with respect to Claim 1?
A. They would.
Q. Is it your opinion that Claim 1 is obvious over the combination of Faria-Urbina and the '793 patent?
A. Yes.
MR. DAVIES: Can we please go to Claim 17.
BY MR. DAVIES:
Q. And, Dr. Channick, why did the documents that we looked at provide a POSA with a reasonable expectation of success with respect to Claim 1?

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A. Because we have retrospective, which is real world, data showing a number of patients getting improvements in exercise capacity in exactly the disease that's in the claim, PH-ILD, and showing that giving it to these patients is safe.
So certainly, although there's not a certainty of success, this is plenty of information, in my opinion, for a POSA to say this is a reasonable expectation of success.
Q. Is it your opinion that the combination of Faria-Urbina and the '793 patent render Claim 17 obvious?
A. Yes.
MR. DAVIES: Let's look at Claim 17.
BY MR. DAVIES:
Q. What additional limitation beyond Claim 1 is provided in Claim 17?
A. So this is specifically saying that the six-minute walk distance of the patient by -- must improve by at least 10 meters after eight weeks of administration.
Q. Do you recall that we already looked at the supplemental tables in S3 and S4 in Faria-Urbina? Do you recall that?
A. Yes.
Q. And based on those tables, did the patients show an average increase in six-minute walk distance of at least

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10 meters after eight weeks of administering treprostinil?
A. Yes.
Q. Do you recall that Dr. Nathan opined that Claim 17 requires that this improvement in patients six-minute walk distance after eight weeks must occur at eight weeks exactly?
A. Yes.
Q. Do you agree with his opinion?
A. No.
Q. And why not?
A. Well, for one thing, we don't measure something to the day of eight weeks on a specific day. And when something says after eight weeks, it means eight weeks or beyond. Or else it would say at eight weeks of administering. It says after eight weeks.
Q. Would a POSA have a reasonable expectation of success with respect to Claim 17 with the combination of Faria-Urbina 2018 for the claim -- for claims -- sorry. Let me do that again.
Would a POSA have a reasonable expectation of success with respect to Claim 17 for the combination of Faria-Urbina and the '793 patent?
A. Yes.
Q. And is the basis for that opinion the same as you've already expressed in the context of the Claim 1?

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A. It is.
MR. DAVIES: Can we go to Claim 14.
BY MR. DAVIES:
Q. Is it your opinion that Claim 14 is obvious in view of Faria-Urbina and the '793 patent?
A. Yes.
Q. Does Claim 14 -- well, what claim does Claim 14 depend from?
A. Claim 11.
Q. And what additional limitations does Claim 11 require beyond Claim 1?
A. That the administration is done by pulsed inhalation device.
Q. What additional limitation does Claim 14 require beyond Claim 11?
A. A dry powder inhaler.
Q. Are you aware the Court construed a pulsed inhalation device in this case to be a device that provides for noncontinuous inhaled drug delivery?
A. Yes.
Q. Did you apply the Court's construction in forming your opinions?
A. I did.
Q. With the Court's construction, do you consider a dry powder inhaler to be a pulsed inhalation device?

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A. I do.
Q. Can we please go to DTX 2, page 20. This is the '793 patent. Based on this description, does the '793 patent disclose a dry powder inhaler and a dry powder for treprostinil?
A. It does.
Q. Do you know whether UTC obtained patent claims in the '793 patent for the use of a dry powder inhaler?
A. Yes.
Q. With treprostinil?
A. Yes.
Q. Is there any additional motivation to combine Faria-Urbina with the '793's disclosure of a dry powder inhaler beyond what we discussed earlier?
A. Yes.
Q. And what would that be?
A. Well, you now have the ability to give a dry powder inhaler which is going to add a level of convenience. We know -- we already have evidence of the benefit for inhaled treprostinil and now we have a method that could add to the convenience and simplicity for the patient.
Q. How was the Tyvaso supplied in the Faria-Urbina 2018 paper?
A. The nebulizer.
Q. It's your opinion that a POSA would be motivated to

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apply the formulation and teaching of Faria-Urbina to the dry powder inhaler of the '793 patent?
A. Definitely.
Q. Were dry powder inhalers in use as of April 2020 for other drugs for the treatment of the airway diseases?
A. Yes, asthma, COPD, those kind of things.
Q. Do you have an opinion regarding a reasonable expectation of success with respect to Claim 14?
A. Yes.
Q. We already looked at Faria-Urbina 2018 and the '793 patent. What do they disclose with respect to the safety of Tyvaso in PH-ILD patients?
A. It demonstrated safety.
Q. Would a nebulizer and a DPI deliver treprostinil through the same inhaled route?
A. Yes.
Q. Does Faria-Urbina 2018 indicate that Tyvaso produced improvements in exercise capacity?
A. It did show that, yes.
Q. Given that, would a POSA have a reasonable expectation of success in combining Faria-Urbina 2018 with the dry powder inhaler in the '793 patent to yield the improvements in exercise capacity in PH-ILD patients in Claim 1?
A. Yes.

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Q. I'm sorry. For Claim 14?
A. Claim 14.
Q. Let me ask that again.
Would a POSA have a reasonable expectation of success in combining Faria-Urbina 2018 with a dry powder inhaler in the '793 patent to yield the improvements in exercise capacity in PH-ILD patients for Claim 14?
A. Yes.
Q. Why?
A. Everything is there to give you that expectation of success from the data showing the benefit, the dry powder inhaler which is going to be delivering inhaled treprostinil in powder formulation and as a POSA one would expect a combination would lead to success.
Q. Can we go to Table S3 of Faria-Urbina.
And what was the increase in the meters in the six-minute walk distance that was observed in the ILD patients in Table 3?
A. 21 meters.
Q. Were those measurements taken after week 8?
A. Yes.
Q. Can we go to Table S4. And Table S4, what was the improvement in meters in six-minute walk distance for these PH CPFE patients?
A. 55 meters.

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BY MR. DAVIES:
Q. Does the 2017 INCREASE protocol say anything about measuring the six-minute walk distance?
A. It does.
Q. What does it tell you about measuring the six-minute walk distance?
A. It says: "The primary outcome measure will be changed in six-minute walk distance measured at peak exposure from baseline to week 16."
Q. And is that the same primary outcome measure required by the INCREASE trial?
A. Yes.
Q. Does that meet the limitation of Claim 1?
A. Yes.
Q. Is it your opinion that following the 2017 protocol necessarily results in the outcomes of Claim 17 of the '327 patent?
A. Yes.
MR. DAVIES: Can we go to Claim 5.
BY MR. DAVIES:
Q. And what does Claim 5 require, Dr. Channick?
A. Again, this is the reduction in plasma NT-proBNP in the patient by at least 200 picograms per milliliter after 8, 12, and 16 weeks.
MR. DAVIES: And can we go to Table 2 on page 8

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of *The New England Journal of Medicine* publication.
BY MR. DAVIES:
Q. Did the INCREASE trial show a reduction in the plasma concentration of NT-proBNP of the patient by at least 200 picograms per mil after 8, 10, or 16 weeks of the administering?
A. Yes.
Q. And what was the average decrease that was observed with 16 weeks?
A. Minus 396 picograms per milliliter.
MR. DAVIES: Can we go to page 11 of the 2017 INCREASE protocol.
BY MR. DAVIES:
Q. Does the 2017 protocol tell you to measure NT-proBNP?
A. Yes.
Q. And so is it your opinion that following the 2017 protocol will result in at least as good outcomes as required by Claim 5 of the '327 patent?
A. It will.
Q. Do you believe it will necessarily and inevitably achieve those results?
A. Yes.
MR. DAVIES: Can we look at Claim 6.
BY MR. DAVIES:
Q. And Claim 6 requires a statistically significant

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reduction in exacerbations of interstitial lung disease.
Do you see that?
A. Yes.
Q. Can we go to page 7 --
A. At least one exacerbation.
Q. At least one exacerbation of underlying lung disease. Thank you, Doctor.
MR. DAVIES: Can we go to page 7 of the *The New England Journal of Medicine* publication.
BY MR. DAVIES:
Q. Did the INCREASE trial show a statistically significant reduction in at least one exacerbation of interstitial lung disease?
A. Yes.
Q. And is it your opinion that following the 2017 protocol will necessarily result in at least as good outcomes as required by Claim 6 of the '327 patent?
A. Yes.
MR. DAVIES: Can we go to Claim 9.
BY MR. DAVIES:
Q. What's required by Claim 9, Doctor?
A. This is the statistically significant improvement in forced vital capacity in the patient after 8, 12, or 16 weeks.
MR. DAVIES: And can we look at page 36 of *The*

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New England Journal of Medicine publication.
BY MR. DAVIES:
Q. Did the INCREASE trial show a statistically significant improvement in percent predicted forced vital capacity in a patient after 8, 12, or 16 weeks of administering?
A. It did.
Q. And where do you see that?
A. On the table that we reviewed earlier where we see .77 percent at eight weeks and 1.07, giving a treatment difference of 1.79 percent and 1.8 percent, both of which are statistically significant improvements in FVC percent predicted.
MR. DAVIES: And can you please go to, now, page 11 of the 2017 increased protocol.
BY MR. DAVIES:
Q. Is the 2017 increased protocol saying anything about measuring FVC?
A. It does.
Q. Is it your opinion that following the 2017 protocol will necessarily result in a statistically significant improvement in percent predicted FVC?
A. It will.
Q. In your opinion, would following the 2017 protocol necessarily result in the Claims 1, 5, 6, 9, and 17 of the

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'327 patent?
A. Yes.
Q. Why?
A. For all the reasons that are stated, that these are measurements described in the protocol, the 2017 protocol, that led to the data and the INCREASE study, which led to the claims in the patent.
Q. Did both the 2017 INCREASE study protocol and the INCREASE study both use the same drug?
A. They do.
Q. Do they both use the same dosing?
A. Yes.
Q. And do they both describe essentially the same PH-ILD population?
A. Yes.
Q. And is it your understanding that the claims of the '327 patent are from the INCREASE study?
A. Yes.
Q. And for that reason, in your opinion, would following the 2017 protocol necessarily result in the outcomes, again, of Claims 1, 5, 6, 9, and 17 of the '327 patent?
A. Yes.
Q. Do you understand that Dr. Nathan argues that the 2017 protocol doesn't inherently anticipate the claims because virtually all of the patients in the INCREASE study

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did not achieve the claimed results?
Do you recall that?
A. Yes.
Q. Do you agree with his opinion?
A. No.
Q. Why not?
A. Because one doesn't need to see virtually all patients achieving the claimed results. That's not what the claims say and that's not what's required, in my understanding and opinion.
Q. Have you ever been involved in a clinical trial were virtually all patients achieved the desired results?
A. No.
Q. In INCREASE did virtually all patients achieve the claimed outcomes?
A. No.
Q. Do you recall the Court's construction of the terms "a" and "the"?
A. Yes.
Q. And how, if at all, did the Court's claim construction of a and the impact your opinion as to whether the claims require virtually all patients to achieve the claimed results?
A. They clearly don't based on that claim construction.
Q. Do you recall that Dr. Nathan opined that the 2017 protocol does not inherently anticipate the asserted claims of the '327 patent because it doesn't disclose any results?
A. Correct.
Q. Do you agree with that opinion?
A. No.
Q. Why not?
A. Because it doesn't have to disclose any results to anticipate the claim.
Q. Do you also recall that Dr. Nathan opined that the 2017 protocol would not inherently anticipate the claims because he alleges there was skepticism that treating PH-ILD with any drug would work?
A. I did hear that, yes.
Q. Do you agree with that opinion?
A. No.
Q. And why not?
A. Because, as I think we've sort of laid out, if anything, it was the opposite. There was this optimism and expectation that it would work.
Q. Do you have any understanding as to whether skepticism matters in the context of inherent anticipation?
A. It does not.
Q. Do you agree that there was a lot of skepticism in the field regarding whether treprostinil would be effective in PH-ILD patients?

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A. I wouldn't characterize it as skepticism, no.
Q. I'd like now to turn to your opinions on the written description of Claim 9.
MR. DAVIES: Could we please bring up Claim 9.
BY MR. DAVIES:
Q. And do you see the claim's reference to the method of Claim 1?
A. Yes.
Q. And what do you understand that to mean?
A. This says: "Statistically significant improvement of forced vital capacity, or FVC, of the patient at 8, 12, or 16 weeks."
Q. And that's the additional limitation that's required by Claim 9; correct?
A. Yes.
Q. And Claim 9 also includes the limitations of Claim 1?
A. Correct.
Q. And would that also include the PH-ILD patient population of Claim 1?
A. Yes.
Q. Do you have an opinion as to whether there is sufficient written description support for the statistically significant improvement in forced vital capacity limitation of Claim 9?
A. I do have an opinion.

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Claim 9?
A. No.
Q. I want to look at Tables 2 and 3 now in the patent. Is the FVC data in Table 2 and 3 of the patent presented in the same way as Table 1?
A. Yes.
Q. Did you review Table 2 in forming your opinions?
A. I did.
Q. What is shown in Table 2?
A. Table 2 is a subpopulation of the whole population. This is the group of patients who have IIP or idiopathic interstitial pneumonia.
Q. How does the IIP population shown in Table 2 relate to the full scope of the ITT population?
A. It's about half of it.
Q. How do you know that?
A. We can look at the end and compare them.
Q. Which end are you looking at specifically, Doctor?
A. If we look at the column N, we can see 58 inhaled treprostinil and 71 in placebo, so that's 129.
Q. Is that roughly half of what we saw for the intent to treat population in that same column?
A. Yeah.
Q. Are all the patients in Table 2 also the part of the patients in Table 1?

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A. Yes.
Q. Can we turn to Table 3 of the '327 patent. Did you review Table 3 in forming your opinions?
A. Yes.
Q. What's shown in Table 3?
A. Table 3 is a subgroup of the subgroup. So these are the patients with the idiopathic interstitial pneumonia who had IPF or idiopathic pulmonary fibrosis.
Q. About what proportion of the patients within the intent to treat group are represented here in this ITF sub subpopulation?
A. About a third.
Q. And, again, how do you know that?
A. We look at the ends and we can see 31 plus 47. That's 78.
Q. Are there any additional patients shown in Tables 2 or 3 that are not part of the intent to treat patients in Table 1?
A. No.
Q. Can we take a look at Table 10. Did you review Table 10 in forming your opinion?
A. I did.
Q. What data is shown in Table 10?
A. This is really just summary data showing the FVC effect.

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Q. Is this the same FVC data that's also reported in Table 1?
A. It is.
Q. So like Table 1, were there any statistically significant improvements in absolute FVC results reported in Table 10?
A. No.
Q. And how did Table 10 impact your opinion as to whether the inventors possessed the full scope of Claim 9?
A. It confirmed my opinion.
Q. And considering all the tables that we've looked at, what is your opinion regarding whether the inventors had the full possession of the full scope of a statistically significant improvement in percent predicted FVC of Claim 9?
A. They did not have the full scope of Claim 9.
Q. Why not?
A. Because they did not have FVC absolute and the claim encompasses both percent predicted and absolute.
Q. How did the data in the tables that we've looked at impact your opinion as to whether the inventors possessed the full scope of Claim 9 which requires a statistically significant improvement in FVC results?
A. It confirms my opinion.
Q. Which table would a POSA look to for information regarding the full scope of PH-ILD patients in Claim 9?

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A. The table containing the full patient cohort.
Q. And, again, did Tables 2 and 3 add additional support to Claim 9 beyond Claim 1?
A. No.
Q. Table 1. I apologize.
What is your opinion as to whether the patent conveys the inventors had possession of the full scope of the statistically significant improvement in FVC PH-ILD patients limitation in Claim 9?
A. They do not.
MR. DAVIES: I have no further questions at this time, Your Honor.
THE COURT: All right. Cross-examination.
MR. DAVIES: I apologize, Your Honor, one thing. I ask that DTX 348 be entered.
MR. CARSTEN: Sorry. What is that, Mr. Davies?
MR. DAVIES: DTX 348.
MR. CARSTEN: No objection, Your Honor.
THE COURT: Admitted without objection.
(Thereupon, Defendant's Exhibit DXT 348 was admitted.)
THE COURT: All right. Thank you, Mr. Davies.
MR. CARSTEN: We've got binders, Judge. May I approach?
THE COURT: Yes.

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MR. CARSTEN: Thank you. May I proceed, Your Honor?
THE COURT: Yes.
MR. CARSTEN: Once I have a binder prepared, I'll hand it up to the clerk. There's one missing.
CROSS-EXAMINATION
BY MR. CARSTEN:
Q. Good afternoon, Dr. Channick.
A. Good afternoon.
Q. I handed you two binders. One of them has exhibits we may be referring to during our examination this afternoon. The other has your depositions and your expert reports in them, okay?
A. Okay.
Q. Let's do a little table setting, if we could. You offered opinions on three defenses this afternoon; correct?
A. Yes.
Q. You've offered an opinion on written description pertaining to Claim 9 of the '327 patent; correct?
A. Yes.
Q. Anticipation by the DTX 008 document and that pertains to Claims 1, 5, 6, 9, and 17; correct?
A. Yes.
Q. And then you've offered obviousness opinions over a combination of Faria-Urbina 2018 and the '793 patent for

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Claims 1, 14, and 17; correct?
A. Yes.
Q. And Faria-Urbina 2018, Saggar 2014, and the '793 patent for Claims 5, 6, and 9; correct?
A. Yes.
Q. I'm sorry. Did you hear the question, sir?
A. I answered it.
Q. You answered it and the answer to that was yes; right?
A. It was.
Q. There's a fourth defense at issue in this case, prior sale. You're not offering opinions on that; correct?
A. Correct.
Q. Dr. Channick, let's talk a little bit about the disease of ILD. You published on PH-ILD before; right?
A. Yes.
Q. About ten papers?
A. About.
Q. And in your experience, you understand that ILD does not always cause pulmonary hypertension; correct?
A. Correct.
Q. In fact, you'd agree that at the time of, for example, idiopathic pulmonary fibrosis diagnosis, pulmonary hypertension is present in about 8 to 15 percent of patients; correct?

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A. That range, yes.
Q. So not all ILD causes pulmonary hypertension; right?
A. Right.
Q. And pulmonary hypertension can increase if it presents in ILD patients; correct?
A. I don't understand that question.
Q. The pulmonary -- the level of pulmonary hypertension experienced by a patient can increase over time with an ILD patient; correct?
A. With any patient, yes.
Q. Now, let's turn to your anticipation opinions, if we might.
Now, you testified, as I recall, that the patient population was a little different in that in the INCREASE had broader -- INCREASE study had broader parameters than that set forth in the DTX 008 document; correct?
A. A little broader, yes.
Q. And then with respect to the dosage, you said they're the same; is that right?
A. Yes.
Q. And, in fact, you created a demonstrative, didn't you, and presented that through your counsel on your direct examination; isn't that right?
A. That is correct.

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Q. Okay. And what this demonstrative 3.9 depicts is on the left side is a passage from DTX 008, page 10, and on the other side, the right side, is page DTX 363, page 3; is that right?
A. Yes.
Q. And DTX 008, that's that printout from the ClinicalTrials.gov database; right?
A. Yes.
Q. And the 363, that's the increase -- the actual increase protocol; right?
A. Wait, you mean the one on the right, my right?
Q. The one on the right.
A. Yeah, that's the publication.
Q. Right, the increased publication from the --
A. You said protocol publication.
Q. *New England Journal of Medicine*; right?
A. Correct.
Q. And here, if you look, it says: "The active treprostinil for inhalation solution .6 milligrams per milliliter delivered via an ultrasonic nebulizer."
And you've got the same thing or similar things highlighted on the right; is that correct?
A. Yes.
Q. "Which emits a dose of approximately 6 micrograms per breath."

EXHIBIT F

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

UNITED THERAPEUTICS CORPORATION, Plaintiff, v. LIQUIDIA TECHNOLOGIES, INC., Defendant.	C.A. No. 1:23-cv-00975-RGA **HIGHLY CONFIDENTIAL**

DEFENDANT LIQUIDIA TECHNOLOGIES, INC.'S FIRST AMENDED INVALIDITY CONTENTIONS

Co. v. Teva Pharms. Int'l GmbH, 8 F.4th 1331, 1344 (Fed. Cir. 2021); *see also Hoffman-La Roche Inc. v. Apotex Inc.*, 748 F.3d 1326, 1331 (Fed. Cir. 2014) ("Conclusive proof of efficacy is not necessary to show obviousness. All that is required is a reasonable expectation of success.").

A. Asserted Claims 1–11 and 14–19 of the '327 Patent Are Invalid for Obviousness-Type Double Patenting over the Claims of the '793 Patent

Asserted Claims 1-11 and 14-19 of the '327 patent are invalid for obviousness-type double patenting over the claims '793 patent, which is assigned to UTC. "Obviousness-type double patenting is a judge-made doctrine that prevents an extension of the patent right beyond the statutory time limit. It requires rejection of an application claim when the claimed subject matter is not patentably distinct from the subject matter claimed in a commonly owned patent." *In re Berg*, 140 F.3d 1428, 1431–32 (Fed. Cir. 1988). If the claims at issue are not patentably distinct from the earlier reference claims, the claims at issue are invalid. *Sun Pharm. Industries, Ltd. v. Eli Lilly and Co.*, 611 F.3d 1381, 1384–85 (Fed. Cir. 2010). Obviousness-type double patenting applies because the '327 and '793 patents are commonly owned by UTC and the claims of the '327 patent are not patentably distinct from those of the earlier-expiring, and invalid, '793 patent. Moreover, the '793 patent is in a different patent family so the safe harbor provision pursuant to 35 U.S.C. § 121 does not apply, and UTC has not filed a terminal disclaimer for the '327 patent disclaiming the portion of the patent term beyond the expiration of the '793 patent. This deficiency cannot be cured by filing a terminal disclaimer, because the '793 patent has been ruled invalid.

1. Claim 1 of the '327 Patent is Invalid for Obviousness-type Double Patenting Over the '793 Patent

Asserted Claim 1 discloses "A method of improving exercise capacity in a patient having pulmonary hypertension associated with interstitial lung disease, comprising administering by inhalation to the patient having pulmonary hypertension associated with interstitial lung disease an effective amount of at least 15 micrograms up to a maximum tolerated dose of treprostinil or a

Contentions. Discovery and Liquidia's investigation are ongoing, and Liquidia reserves the right to modify and/or supplement its First Amended Invalidity Contentions.

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CERTIFICATE OF SERVICE

I certify that I caused copies of the foregoing document to be served on July 16, 2024 upon the following in the manner indicated:

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EXHIBIT G


US011826327B2

(12) **United States Patent**
Peterson et al.

(10) **Patent No.: US 11,826,327 B2**
(45) **Date of Patent: Nov. 28, 2023**

(54) **TREATMENT FOR INTERSTITIAL LUNG DISEASE**

(71) Applicant: **United Therapeutics Corporation**, Silver Spring, MD (US)

(72) Inventors: **Leigh Peterson**, Hillsborough, NC (US); **Peter Smith**, Durham, NC (US); **Chunqin Deng**, Chapel Hill, NC (US)

(73) Assignee: **United Therapeutics Corporation**, Silver Spring, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 263 days.

(21) Appl. No.: **17/233,061**

(22) Filed: **Apr. 16, 2021**

(65) **Prior Publication Data**

US 2021/0330621 A1 Oct. 28, 2021

Related U.S. Application Data

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(51) **Int. Cl.**

A61K 31/192	(2006.01)
A61P 9/12	(2006.01)
A61K 9/00	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/192*** (2013.01); ***A61K 9/0075*** (2013.01); ***A61K 9/0078*** (2013.01); ***A61P 9/12*** (2018.01)

(58) **Field of Classification Search**

CPC A61K 31/192

See application file for complete search history.

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(57) **ABSTRACT**

Methods of treating of interstitial lung disease, reducing pulmonary function decline in a subject with interstitial lung disease (ILD), and increasing forced vital capacity (FVC) in a subject suffering from ILD are provided, wherein the methods include administration of treprostinil.

19 Claims, 15 Drawing Sheets

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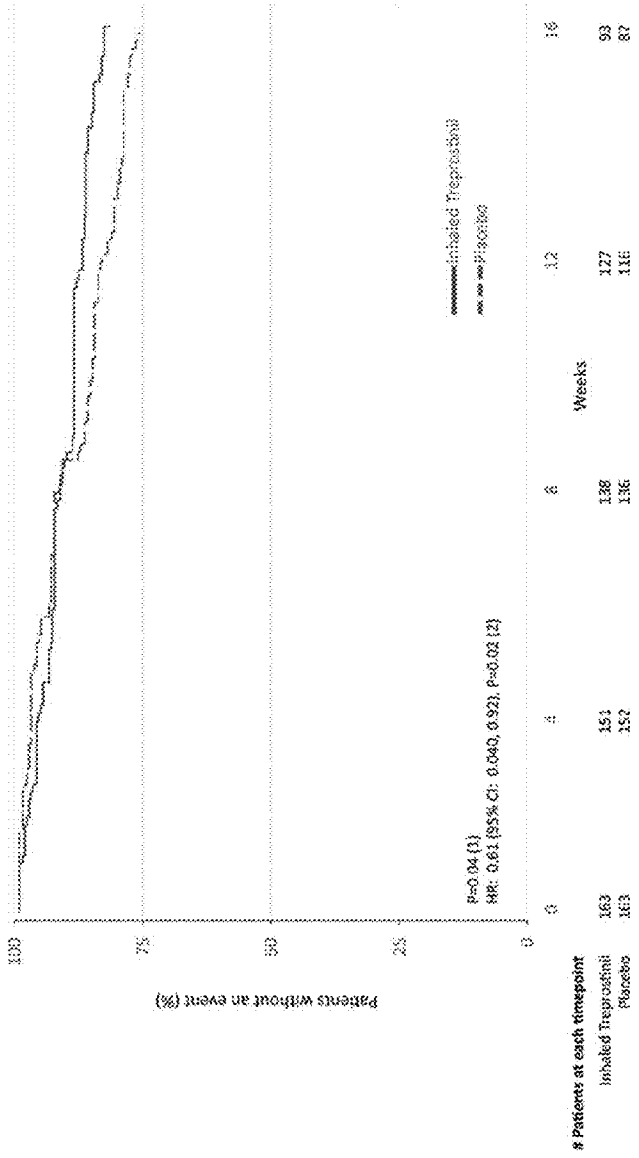
Figure 1


Patients without an event (%)
100
75
50
25
0
Weeks
0
4
8
12
16
Inhaled Treprostinil
Placebo
HR: 0.61 (95% CI: 0.040, 0.92), P=0.02 (2)
P=0.04 (1)
# Patients at each timepoint
Inhaled Treprostinil 163 151 138 127 93
Placebo 163 152 136 116 87

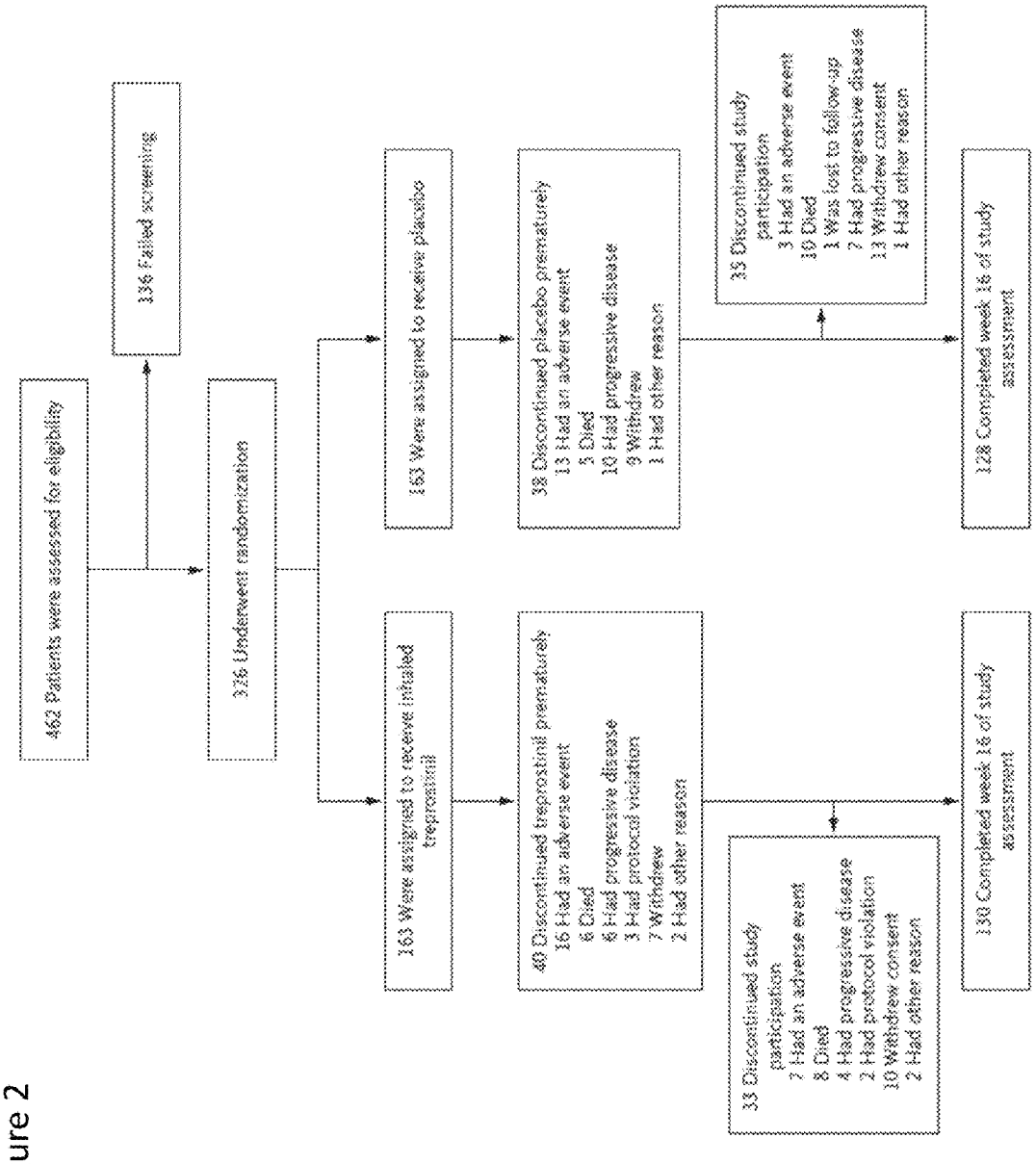

462 Patients were assessed for eligibility
126 Failed screening
336 Underwent randomization
163 Were assigned to receive placebo
38 Discontinued placebo prematurely
13 Had an adverse event
3 Died
10 Had progressive disease
9 Withdrew
1 Had other reason
33 Discontinued study participation
3 Had an adverse event
10 Died
1 Was lost to follow-up
7 Had progressive disease
11 Withdrew consent
1 Had other reason
125 Completed week 16 of study assessment
163 Were assigned to receive inhaled treprostinil
40 Discontinued treprostinil prematurely
16 Had an adverse event
6 Died
6 Had progressive disease
3 Had protocol violation
7 Withdrew
2 Had other reason
33 Discontinued study participation
7 Had an adverse event
8 Died
4 Had progressive disease
2 Had protocol violation
10 Withdrew consent
2 Had other reason
130 Completed week 16 of study assessment

Figure 2

Figure 3

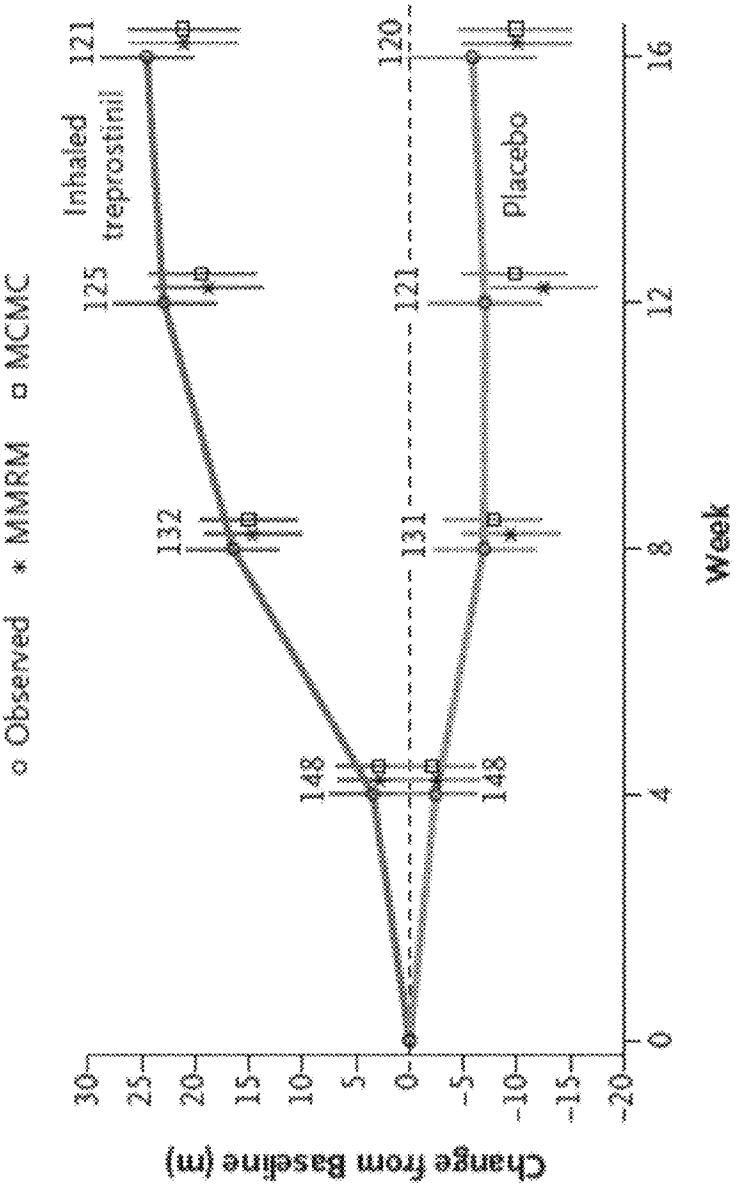

Change from Baseline (m)
Week
Inhaled treprostinil
Placebo
Observed
MMRM
MCMC
121
125
132
148
120
121
131
148
30
25
20
15
10
5
0
-5
-10
-15
-20
0
4
8
12
16

Figure 4

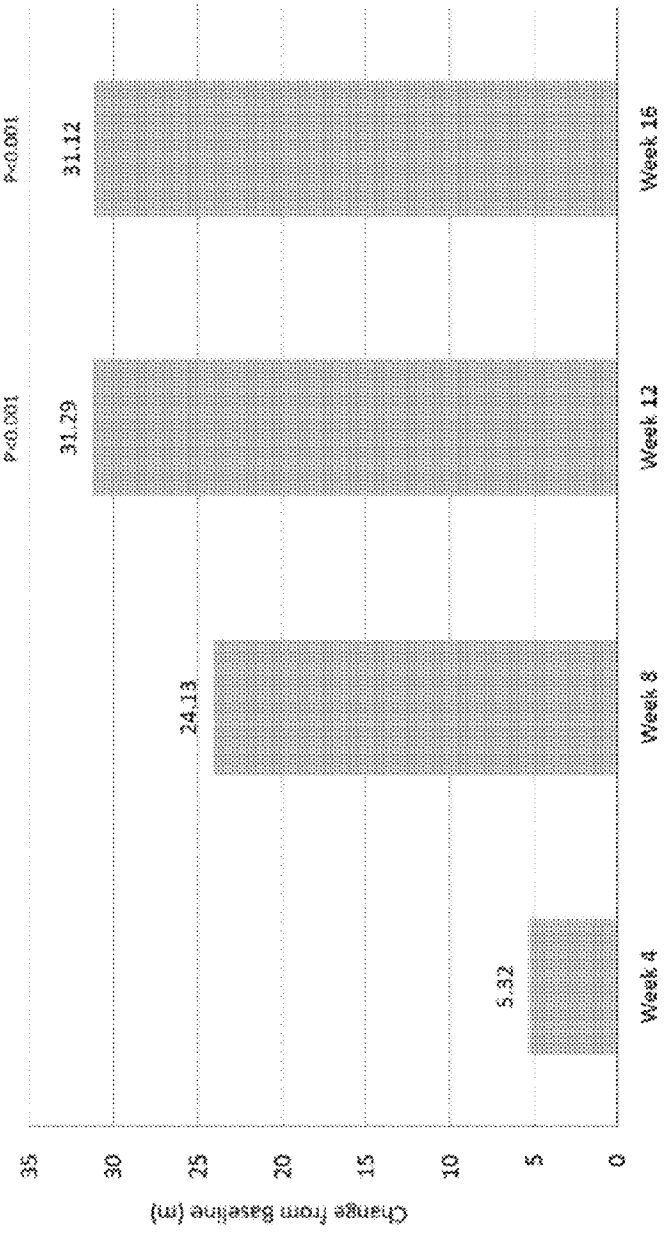

Change from Baseline (m)
35
30
25
20
15
10
5
0
5.32
24.13
31.29
31.12
Week 4
Week 8
Week 12
Week 16

Figure 5

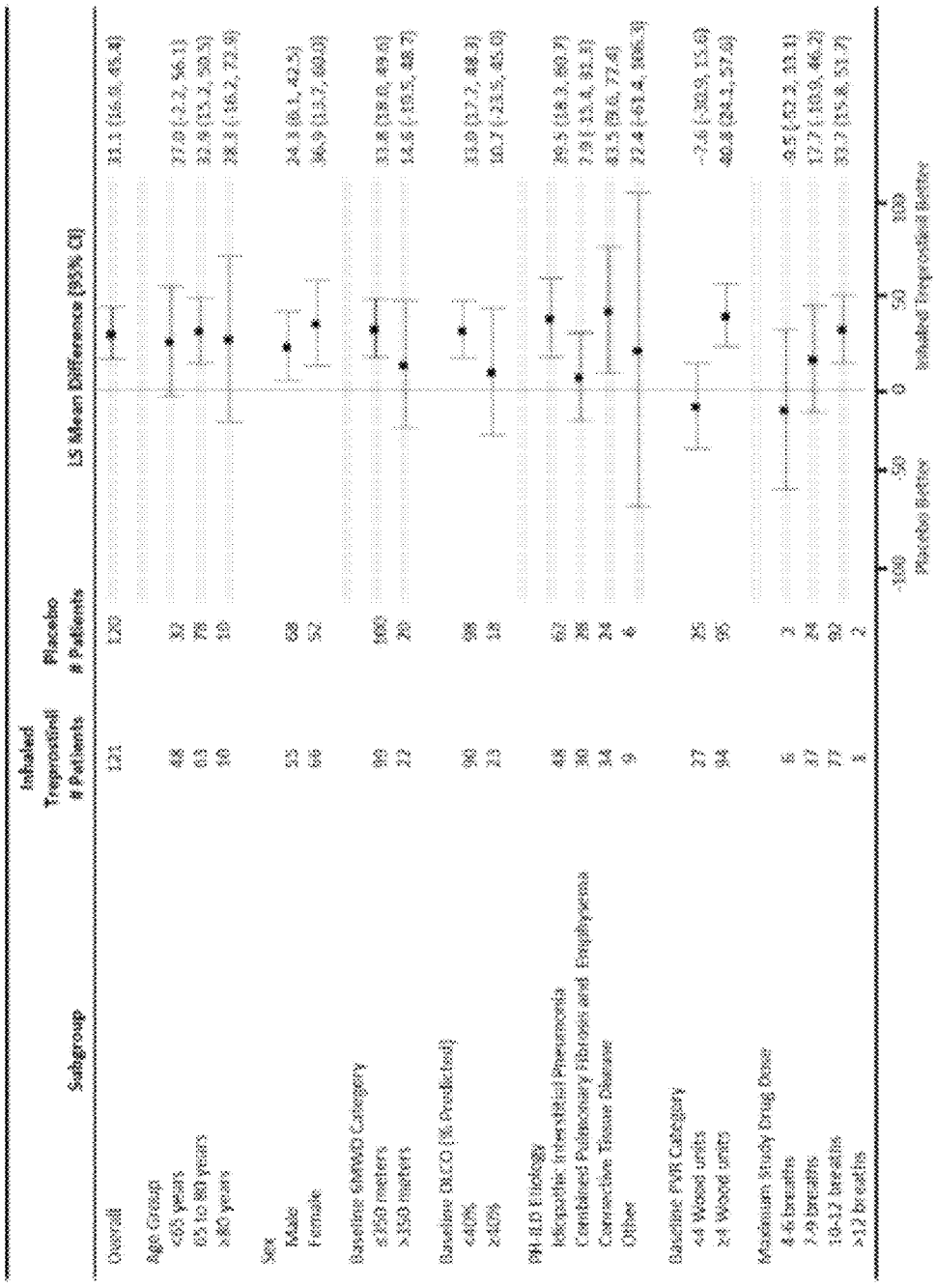

Subgroup
Inhaled Treprostinil # Patients
Placebo # Patients
LS Mean Difference (95% CI)
Overall 121 120 21.1 (-4.6, 46.8)
Age Group
<65 years 48 50 27.0 (2.2, 56.1)
65 to 80 years 63 58 22.8 (-3.2, 50.1)
>80 years 10 12 28.3 (-10.2, 72.8)
Sex
Male 53 58 24.3 (-0.1, 42.5)
Female 68 62 20.6 (-3.7, 60.0)
Baseline 6MWD Category
≤250 meters 90 96 23.8 (2.8, 49.6)
>250 meters 32 24 14.6 (-19.5, 48.7)
Baseline DLCO (% Predicted)
<40% 90 88 23.0 (2.7, 48.2)
≥40% 33 32 10.7 (-23.5, 45.0)
PH-ILD Etiology
Idiopathic Interstitial Pneumonia 48 50 29.5 (-8.2, 60.9)
Combined Pulmonary Fibrosis and Emphysema 38 38 7.9 (-23.4, 32.3)
Connective Tissue Disease 34 24 43.5 (9.0, 77.6)
Other 9 8 22.4 (-61.4, 106.3)
Baseline PVR Category
<4 Wood units 27 26 -7.4 (-30.9, 15.6)
≥4 Wood units 94 94 40.8 (24.1, 57.6)
Hospitalization Study Drug Dose
≤6 breaths 6 7 -9.5 (-52.3, 33.1)
7-9 breaths 22 24 17.7 (-10.0, 46.2)
10-12 breaths 77 82 23.7 (-3.8, 51.2)
≥13 breaths 1 2
-100 -50 0 50 100
Placebo Better
Inhaled Treprostinil Better

Figure 6

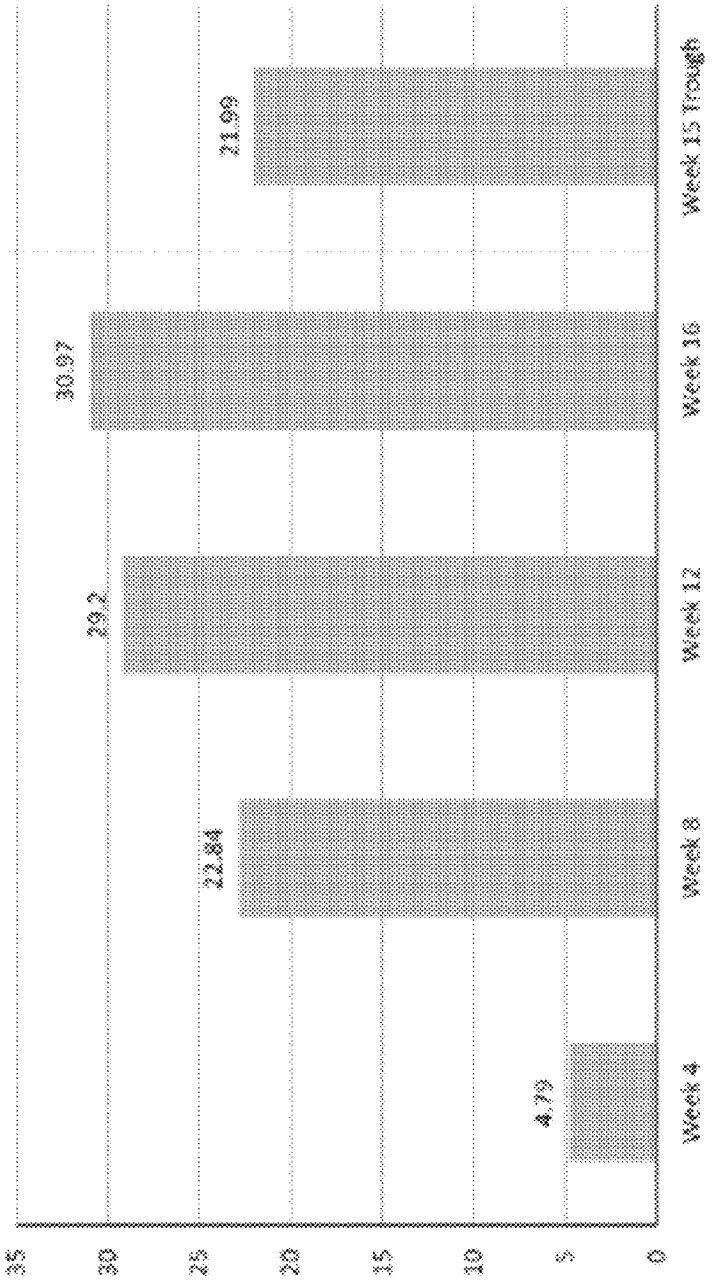

35
30
25
20
15
10
5
0
4.79
22.84
29.2
30.97
21.99
Week 4
Week 8
Week 12
Week 16
Week 15 Trough

Figure 7

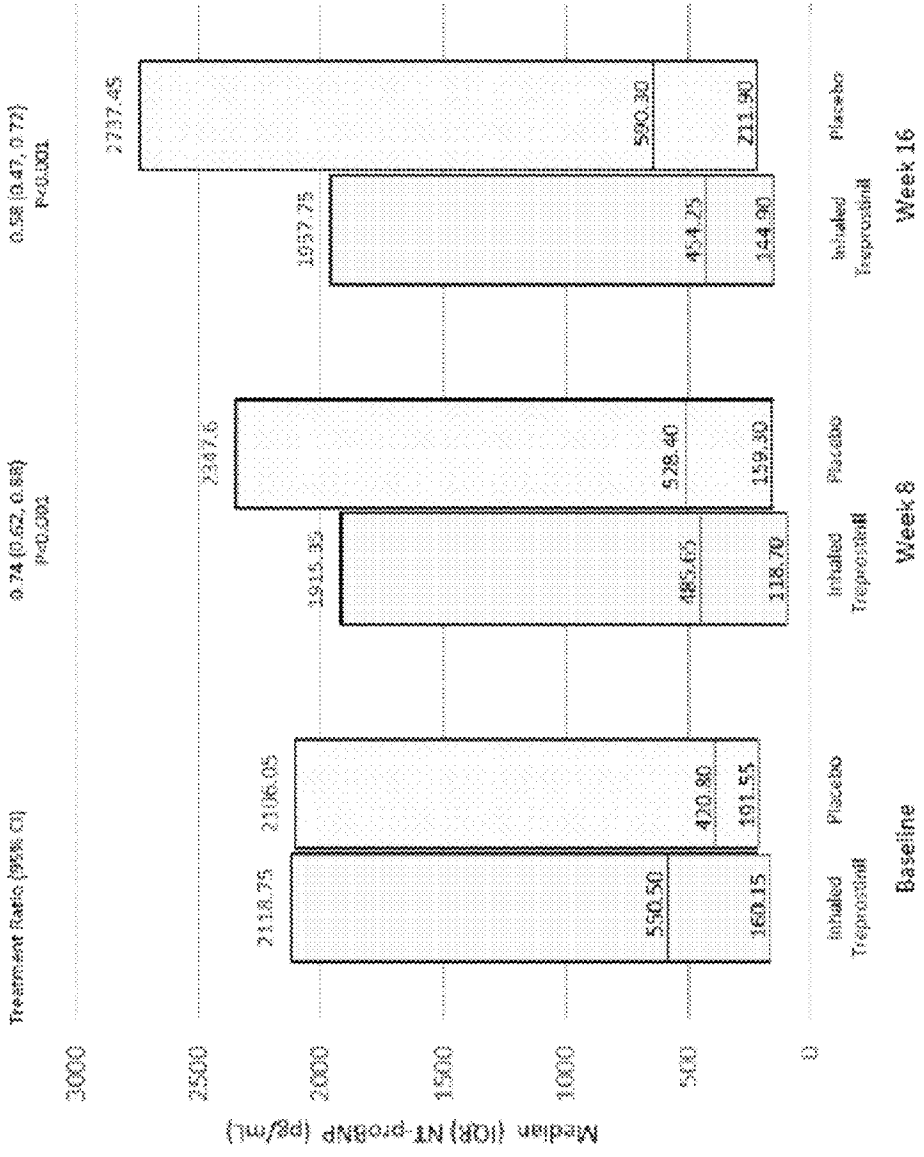
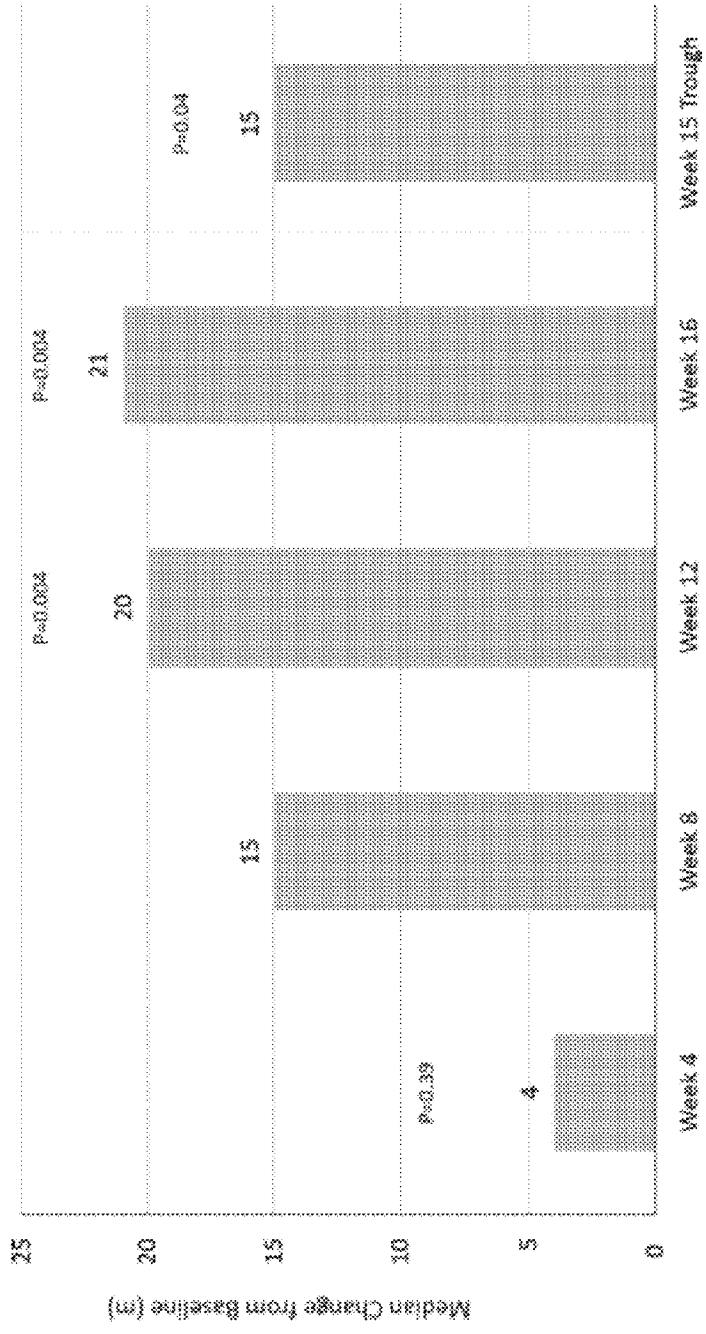

Median (IQR) NT-proBNP (pg/mL)
3000
2500
2000
1500
1000
500
0
Treatment Ratio (95% CI)
Baseline
Week 8
Week 16
Inhaled Treprostinil
Placebo
2118.75
590.50
160.15
2106.05
420.80
191.55
1915.30
485.65
118.70
2387.6
528.40
139.30
1657.75
454.25
144.90
2737.45
590.30
211.90
0.74 (0.62, 0.88) P<0.001
0.58 (0.47, 0.72) P<0.001

Figure 8


Median Change from Baseline (m)
25
20
15
10
5
0
Week 4
4
P=0.39
Week 8
15
Week 12
20
P=0.004
Week 16
21
P=0.004
Week 15 Trough
15
P=0.04

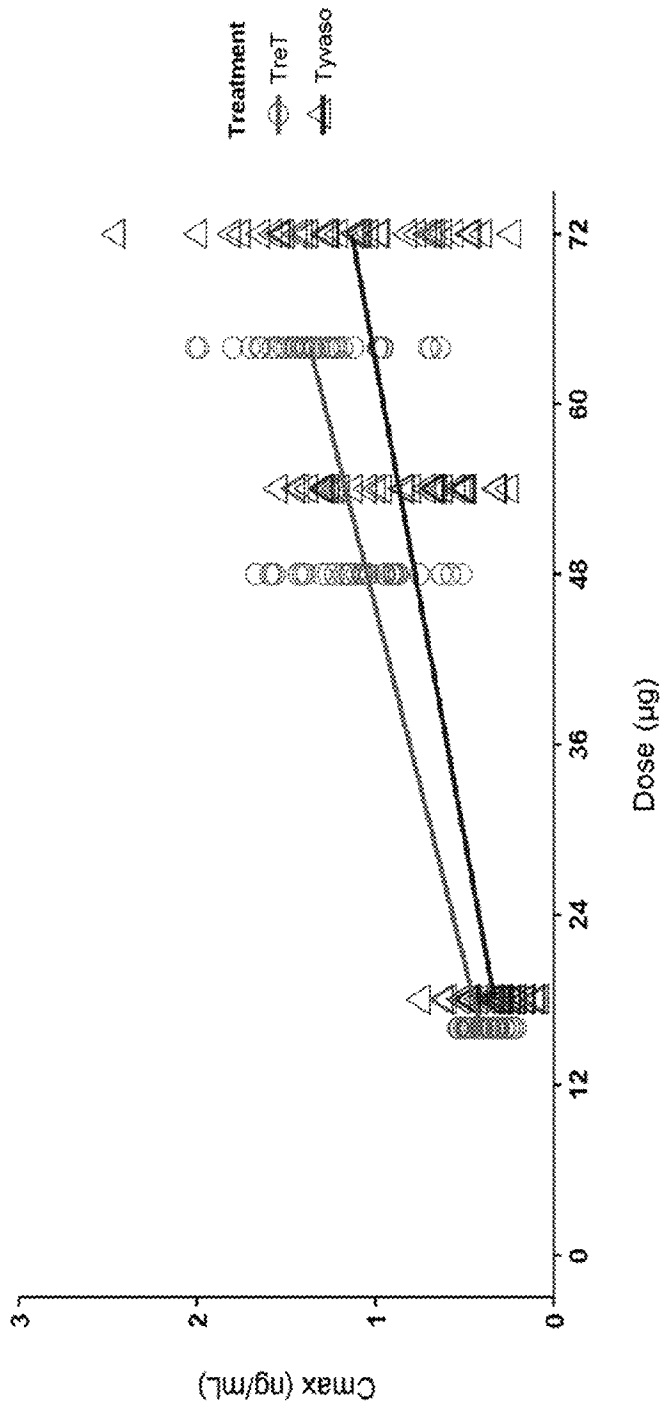

Treatment
TreT
Tyvaso
Cmax (ng/mL)
0
1
2
3
Dose (µg)
0
12
24
36
48
60
72

Figure 9

Figure 10

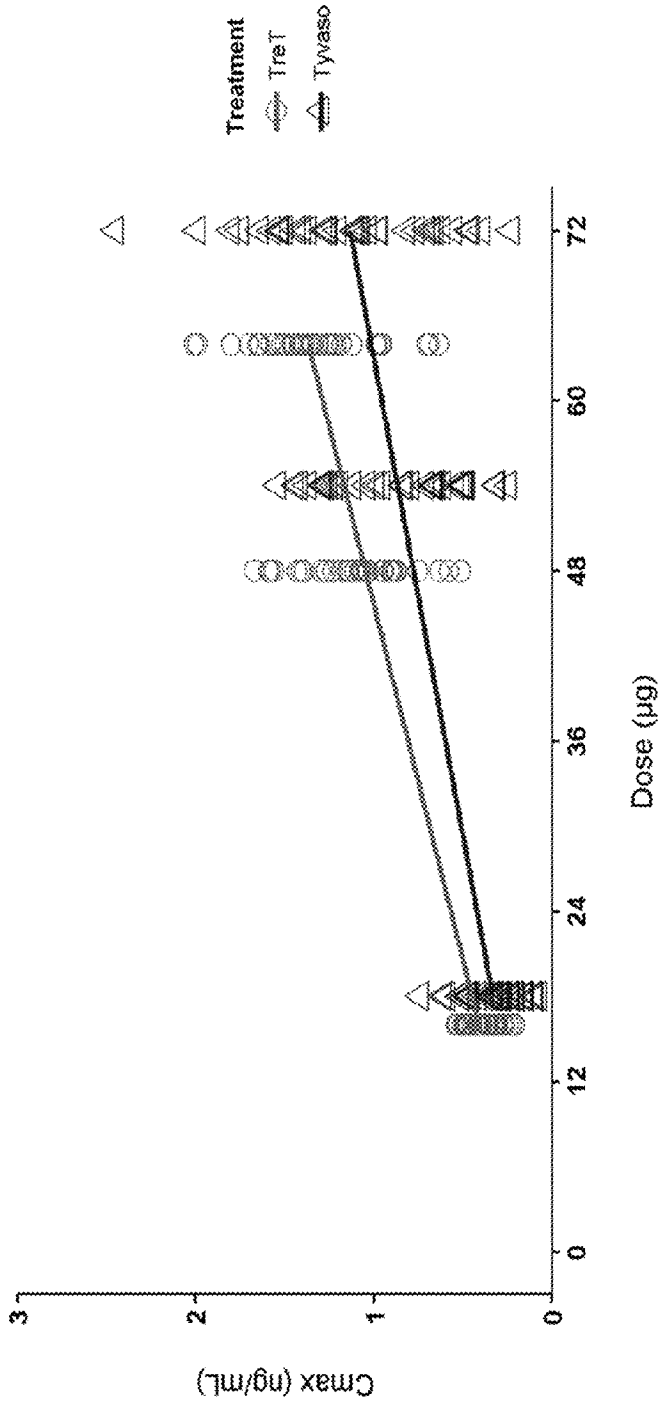

Treatment
TreT
Tyvaso
Cmax (ng/mL)
0
1
2
3
Dose (µg)
0
12
24
36
48
60
72

Figure 11

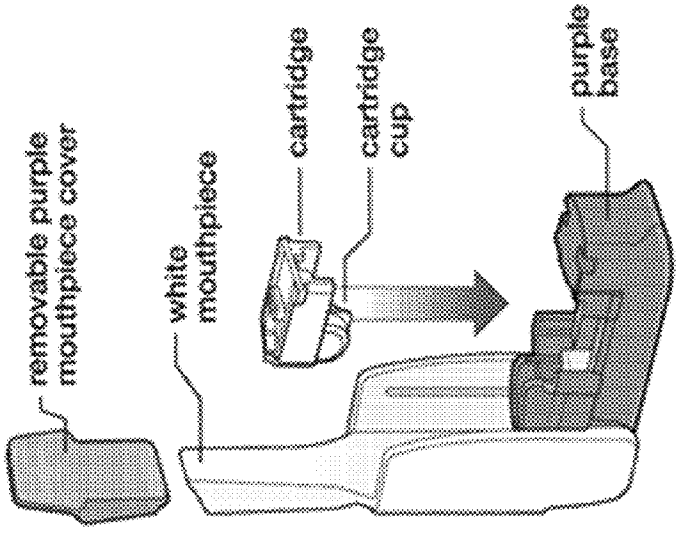

removable purple mouthpiece cover
white mouthpiece
cartridge
cartridge cup
purple base

Figure 12

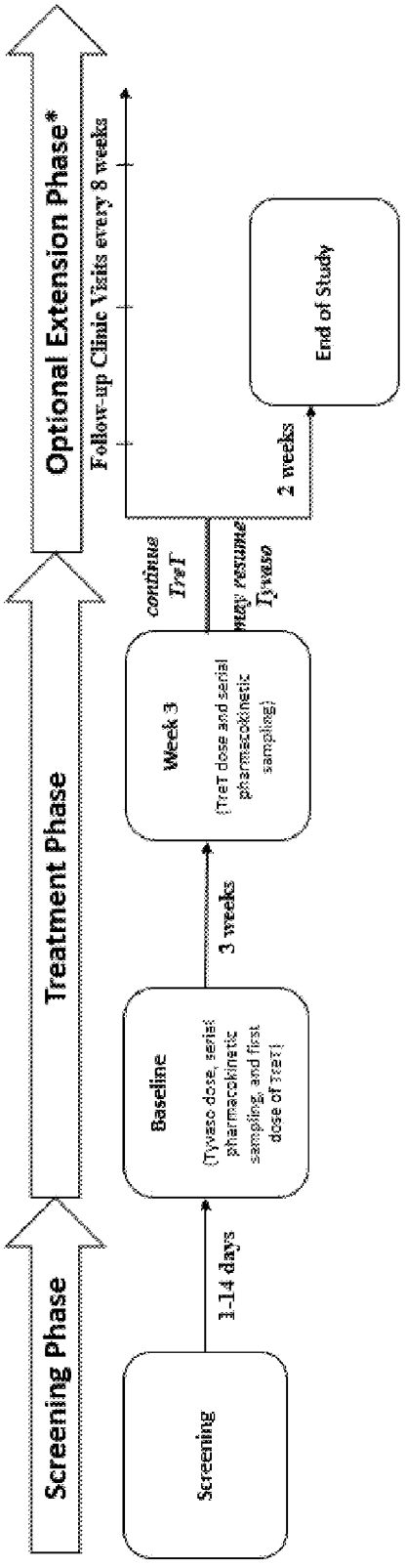

Screening Phase
Treatment Phase
Optional Extension Phase*
Follow-up Clinic Visits every 8 weeks
Screening
1-14 days
Baseline
(Tyvaso dose, serial pharmacokinetic sampling, and first dose of TreT)
3 weeks
Week 3
(TreT dose and serial pharmacokinetic sampling)
continue TreT
may resume Tyvaso
2 weeks
End of Study

Figure 13

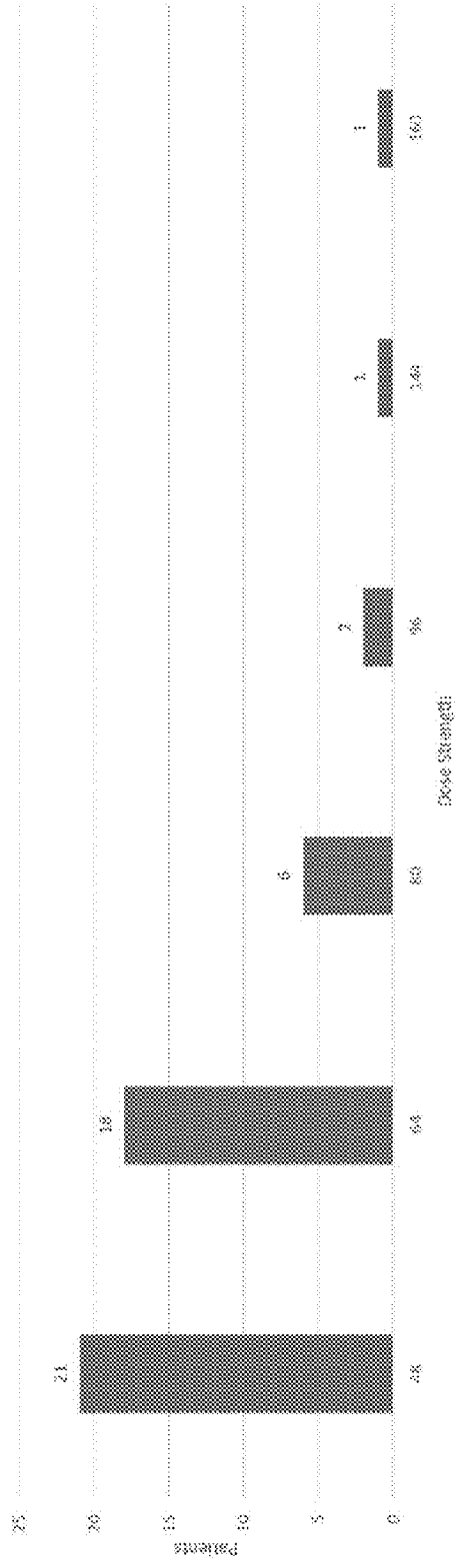
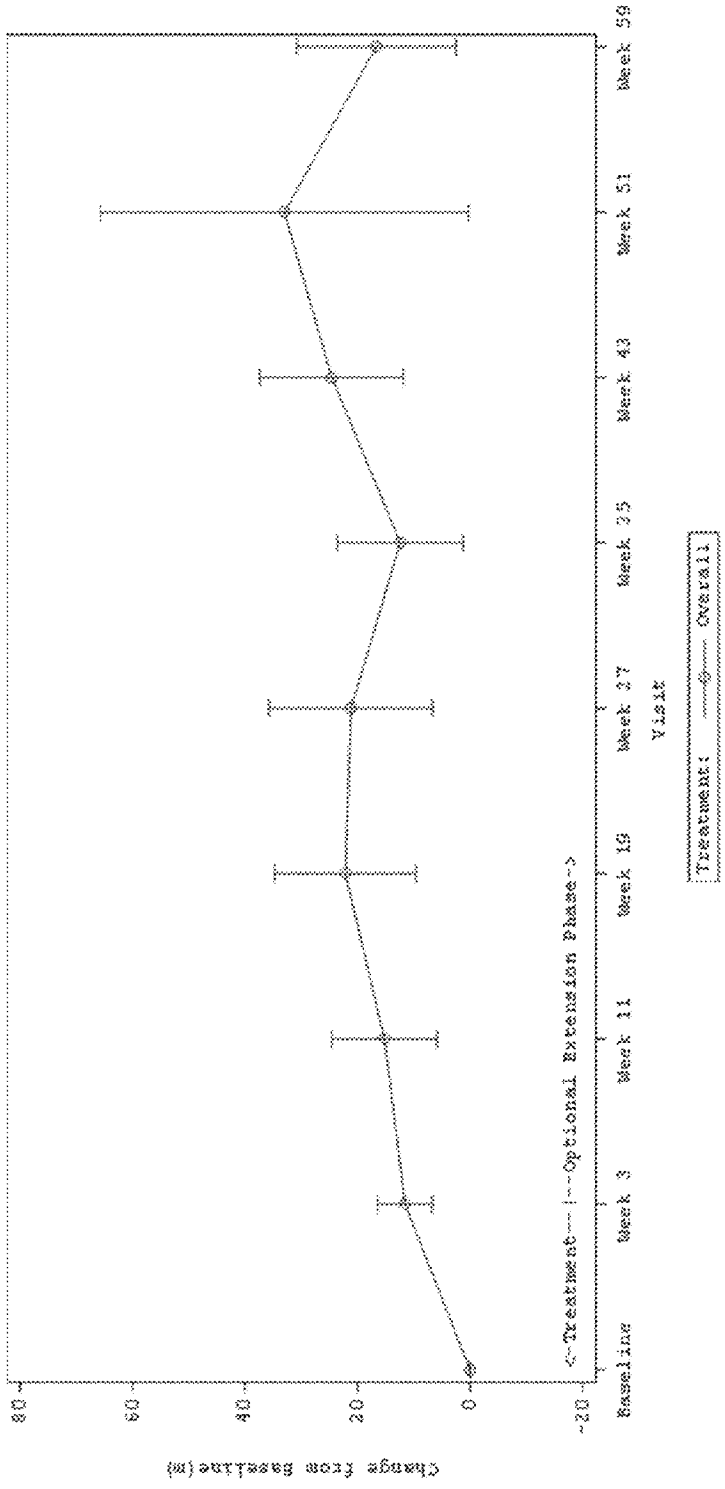


Figure 14


Change from Baseline(%)
80
60
40
20
0
-20
Baseline
Week 3
Week 11
Week 19
Week 27
Week 35
Week 43
Week 51
Week 59
<--Treatment--|--Optional Extension Phase-->
Visit
Treatment: Overall

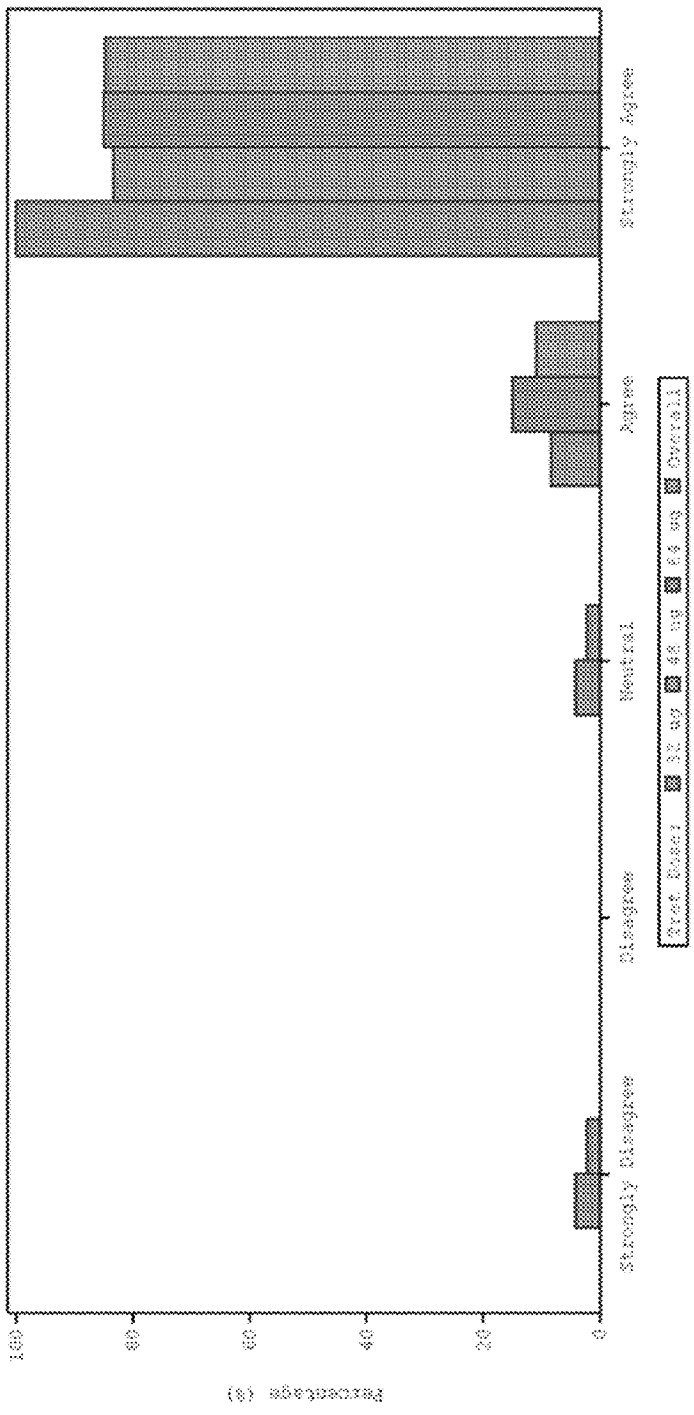

Strongly Agree
Agree
Neutral
Disagree
Strongly Disagree
Overall
100
80
60
40
20
0

Figure 15

TREATMENT FOR INTERSTITIAL LUNG DISEASE

RELATED APPLICATIONS

The present application claims priority to U.S. provisional application No. 63/011,810 filed Apr. 17, 2020 and U.S. provisional application No. 63/160,611 filed Mar. 12, 2021, each of which is incorporated herein by reference in its entirety.

FIELD

The present application generally relates to methods of treating a disease with prostacyclins and more particularly, to treating a disease with treprostinil.

BACKGROUND

Interstitial lung disease (ILD), or diffuse parenchymal lung disease (DPLD), is a group of lung diseases affecting the interstitium (the tissue and space around the alveoli, including air sacs of the lungs). It concerns alveolar epithelium, pulmonary capillary endothelium, basement membrane, and perivascular and perilymphatic tissues. It may occur when an injury to the lungs triggers an abnormal healing response. Such abnormal response may result in idiopathic pulmonary fibrosis (IPF). Currently, two drugs are approved by FDA for treatment of IPF, which is the most common form of PF: nintedanib and pirfenidone. The average rate of survival for someone with interstitial lung disease is currently between 3 and 5 years (Meyer et al., 2017). There exists a need for the identification of new pharmaceutical treatments for ILD.

SUMMARY

In one aspect, a method of treating a pulmonary hypertension due to a condition which is selected from a chronic lung disease, hypoxia and a combination thereof, comprises administering to a subject having the pulmonary hypertension due to the condition selected from a chronic lung disease, hypoxia and a combination thereof an effective amount of treprostinil, a prodrug thereof or a pharmaceutically acceptable salt thereof.

In one aspect, a method of treating interstitial lung disease (ILD) in a subject in need thereof is provided, comprises administering to the subject a therapeutically effective amount of treprostinil, a prodrug, salt, or ester thereof. In an embodiment, the subject has pulmonary hypertension associated with ILD.

In one aspect, a method of reducing pulmonary function decline in a subject with ILD is provided, comprises administering to the subject treprostinil, a prodrug, salt, or ester thereof.

In one aspect, a method of increasing forced vital capacity (FVC) in a subject suffering from ILD is provided, comprises administering to the subject treprostinil, a prodrug, salt, or ester thereof. In some embodiments, administration of treprostinil, a prodrug, salt, or ester thereof may result in an increase of FVC of at least 20%, at least 40%, at least 60%, at least 80%, at least 90%, or at least 100% compared to the FVC prior to the start of treatment. The FVC can be assessed prior to the start of treatment and at intervals after the start of treatment. For example, the pre-treatment FVC can be compared to the FVC measured at one week, four weeks, eight weeks, or sixteen weeks after the start of treatment.

In some embodiments, administering an effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may provide an improvement, which may be statistically significant, in forced vital capacity (FVC) in a subject with a condition selected from a chronic lung disease, such as an ILD or IPF and/or hypoxia. For example, the FVC may be higher in a patient subpopulation with the chronic lung disease and/or hypoxia, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug for at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks, or at least 28 weeks or at least 32 weeks, or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks, compared to a patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. For example, the FVC value may be higher by at least 10 ml or at least 15 ml or at least 20 ml or at least 25 ml or at least 30 ml or at least 35 ml or at least 40 ml or at least 45 ml after at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks of the administering in the patient subpopulation with the chronic lung disease and/or hypoxia, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug compared to the patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. In patients with a chronic lung disease, such as interstitial lung disease, and/or hypoxia, an FVC value usually decreases with time when untreated. Thus, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt may increase an FVC value compared to an FVC value before the administering; maintain an FVC value within 5%, 10% or 20% within the FVC value prior to the administering; or reduce a decrease of an FVC value with time compared to a decrease in an FVC value with no administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt, such a decrease in an FVC value when placebo is administered instead of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt.

In some embodiments, the ILD comprises one or more of idiopathic pulmonary fibrosis (IPF), desquamative interstitial pneumonia (DIP), acute interstitial pneumonia (AIP), nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis-associated interstitial lung disease (RB-ILD), cryptogenic organizing pneumonia (COP), lymphoid interstitial pneumonia (LIP), sarcoidosis, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, antisynthetase syndrome, silicosis, asbestosis, occupational lung disease, chronic hypersensitivity pneumonitis, idiopathic interstitial pneumonia (IIP), an autoimmune ILD, lymphangioleiomyomatosis (LAM), Langerhan's cell histiocytosis (LCH), drug associated ILD, vasculitis, granulomatosis, and berylliosis. In some embodiments, the ILD comprises IPF.

In some embodiments, the ILD comprises systemic sclerosis-associated interstitial lung disease (SSc-ILD).

In some embodiments, the ILD was induced from antibiotics, chemotherapy, antiarrhythmic agents, coronavirus disease 2019 (COVID-19), atypical pneumonia, pneumocystis pneumonia, tuberculosis (TB), *Chlamydia trachomatis*, respiratory syncytial virus, or lymphangitic carcinomatosis.

In some embodiments, the subject has one or more of surfactant-protein-B deficiency, surfactant-protein-C deficiency, ABCA3-deficiency, brain lung thyroid syndrome, congenital pulmonary alveolar proteinosis, alveolar capillary dysplasia, mutations in telomerase reverse transcriptase, mutations in telomerase RNA component, mutations in the regulator of telomere elongation helicase 1, and mutations in poly(A)-specific ribonuclease.

In some embodiments, the subject has one or more symptoms of shortness of breath, fatigue, weight loss, dry cough, chest pain, and lung hemorrhage. In some embodiments, after administration the symptom is improved by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, as measured by a medically-recognized technique. In some embodiments, the medically-recognized technique comprises one or more of Modified Medical Research Council (MMRC) Dyspnoea Scale, Modified Borg Dyspnoea Scale (0-10), Chalder Fatigue Scale, weight measurement scale, visual analogue scale (VAS) for cough, King's Brief Interstitial Lung Disease Questionnaire, Leicester Cough Questionnaire (LCQ), Living with IPF (L-IPF, see e.g. Am J Respir Crit Care Med Vol 202, Iss 12, pp 1689-1697, Dec. 15, 2020), computed tomography (CT) scan, X-ray, multiple magnetic resonance imaging (MRI), pulmonary function testing (PFT), spirometry, lung volumes, maximal respiratory pressure, diffusing capacity, oxygen desaturation, and arterial blood gas evaluation.

In some embodiments, treprostinil, a prodrug, salt, or ester thereof is administered in a pharmaceutical composition comprising treprostinil, a prodrug, salt, or ester thereof and a pharmaceutically acceptable carrier or excipient.

In some embodiments, the administration comprises at least one of oral, inhalation, subcutaneous, nasal, intravenous, intramuscular, sublingual, buccal, rectal, vaginal, and transdermal administration. In some embodiments, the administration comprises inhalation. In some embodiments, one inhalation dosing event comprises from 1 to 20 breaths, wherein at least one inhalation dosing event per day is administered.

In some embodiments, the method comprises administration of at least one additional active agent to treat the ILD. In some embodiments, the at least one additional active agent comprises a corticosteroid, mycophenolic acid, mycophenolate mofetil, azathioprine, cyclophosphamide, rituximab, pirfenidone, or nintedanib. In some embodiments, the at least one additional active agent and treprostinil, a prodrug, salt, or ester thereof, are administered via a method selected from the group consisting of (a) concomitantly; (b) as an admixture; (c) separately and simultaneously or concurrently; and (d) separately and sequentially.

In some embodiments, administration is once, twice, thrice, four times, five times, or six times per day. In some embodiments, administration is for a period selected from the group consisting of about 1 day, about 1 day to about 3 days, about 3 days to about 6 days, about 6 days to about 9 days, about 9 days to about 12 days, about 12 days to about 15 days, about 15 days to about 18 days, about 18 days to about 21 days, about 21 days to about 24 days, about 24 days to about 27 days, about 27 days to about 30 days, or about greater than 30 days.

In some embodiments, a method of treating a pulmonary hypertension due to a condition which is selected from a chronic lung disease, hypoxia and a combination thereof, comprises administering to a subject having the pulmonary hypertension due to the condition selected from a chronic lung disease, hypoxia and a combination thereof an effective amount of treprostinil, a prodrug thereof or a pharmaceutically acceptable salt thereof.

In some embodiments, the subject is a human.

FIGURES

FIG. **1** shows a Kaplan-Meier plot of time to exacerbation of underlying lung disease over a 16-week period of treprostinil treatment. CI stands for confidence interval; HR stands for hazard ratio. Subjects who discontinued from the study early had their time to first clinical worsening event censored at their last visit. Subjects who did not experience a clinical worsening event had their time to first clinical worsening event censored at the study termination date. (1) P-value was calculated with log-rank test stratified by baseline 6-minute walk distance category. (2) Hazard ratio, 95% CI, and p-value were calculated with proportional hazards model with treatment and baseline 6-minute walk distance (continuous) as explanatory variables.

FIG. **2** outlines a plan for the clinical study presented in Example 3. Of 462 patients screened for eligibility, 326 patients underwent randomization and received at least one dose of the assigned treprostinil or placebo (included in the intention-to-treat and safety populations). Of the patients who underwent randomization, 40 patients in the treprostinil group and 38 in the placebo group discontinued the assigned regimen prematurely. These patients were not withdrawn from the trial but were encouraged to remain and complete assessments through week 16; 33 patients in the treprostinil group and 35 in the placebo group discontinued trial participation before week 16.

FIG. **3** shows mean change from baseline in peak 6-minute walking distance through week 16 in the clinical study presented in Example 3. Shown are mean (±SE) changes from baseline (dashed line) in peak 6-minute walk distance over the 16-week trial period. The data shown are for patients with available data (observed) as well as for the results of two analysis methods used to account for missing data. The values shown at each data point indicate the number of patients assessed at that time point. The primary analysis used mixed-model repeat-measurement (MMRM) methods, with the assumption that missing data were missing at random. The model included the change from baseline to peak 6-minute walk distance as the dependent variable, with treatment, week, and treatment-by-week interaction as fixed effects, and the baseline 6-minute walk distance as a covariate. A sensitivity analysis for the primary end point was performed with the use of a multiple imputation approach with a multivariate normal imputation model using the Markov chain Monte Carlo (MCMC) method. The imputation model included treatment group, all scheduled visits, patient's sex, and patient's age at randomization. The confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects.

FIG. **4** shows 6-Minute Walk Distance Treatment Effect Using Mixed Model Repeated Measurement Through Week 16. A longitudinal data analysis using mixed model repeated

measurement was also performed to estimate the treatment difference in change in peak 6-minute walk distance at Week 16. The mixed model repeated measurement includes the change from baseline in peak 6-minute walk distance as the dependent variable; treatment, week, and treatment by week interaction as fixed effects; and baseline 6-minute walk distance as a covariate. An unstructured variance/covariance structure shared across treatment groups was used to model the within-subject errors.

FIG. **5** shows Forest Plot on Subgroup Analyses of Peak 6-Minute Walk Distance (meter) at Week 16. 6MWD stands for 6-minute walk distance; CI stands for confidence interval; ILD stands for interstitial lung disease; PH stands for pulmonary hypertension; PVR stands for pulmonary vascular resistance; LS mean differences and their 95% confidence intervals, and p-values are from the mixed model repeated measures. The confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects. For etiology, the "other" category includes chronic hypersensitivity pneumonitis and occupational lung disease.

FIG. **6** shows 6-Minute Walk Distance Treatment Effect Using Multiple Imputation Through Week 16. Multiple imputation approach using a multivariate normal imputation model with the Markov Chain Monte Carlo method. P-values are obtained from 100 multiple imputations using Markov Chain Monte Carlo estimation with ANCOVA model with change from Baseline in 6-minute walk distance as the dependent variable, treatment as fixed effect, and Baseline 6-minute walk distance measurement as a covariate.

FIG. **7** shows NT-proBNP Results by Study Visit (pg/mL). CI stands for confidence interval; IQR stands for interquartile range; NT-proBNP stands for N-terminal pro-brain natriuretic peptide. As displayed above, inhaled treprostinil was associated with a 42% reduction in NT-proBNP compared to placebo at Week 16 (Treatment Ratio 0.58; 95% CI: 0.47, 0.72; P<0.001). Only subjects with a Baseline NT-proBNP measurement are included in this analysis. P-values, estimated treatment ratio, and associated 95% CIs (LS Mean difference expressed as ratio) are obtained from the analysis of covariance with change from baseline in log transformed data in NT-proBNP as the dependent variable, treatment as the fixed effect, and log-transformed baseline NT-proBNP as a covariate. The confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects.

FIG. **8** shows Hodges-Lehmann Estimate of Treatment Effect for 6-Minute Walk Distance Through Week 16. For those subjects who withdrew early due to death, were too ill to walk, or had no 6-minute walk distance measurement due to a clinical worsening event, the 6-minute walk distance was set to 0; for all other withdrawals without a measurement, last observation carried forward was used for imputation. P-values are obtained from nonparametric ANCOVA adjusted for Baseline 6-minute walk distance category.

FIG. **9** is a plot showing a relationship between treprostinil AUC0-5 and dose for Treprostinil Inhalation Powder (TreT) administered by a dry powder inhaler and nebulized treprostinil administered by Tyvaso nebulizer.

FIG. **10** is a plot showing a relationship between treprostinil Cmax and dose for Treprostinil Inhalation Powder (TreT) administered by a dry powder inhaler and nebulized treprostinil administered by Tyvaso nebulizer.

FIG. **11** shows a dry powder inhaler, which has a cartridge with a dose of Treprostinil Inhalation Powder (TreT).

FIG. **12** shows a design of a study of Example 5. During the Optional Extension Phase (OEP), dosing titration is encouraged; the dose of TreT is titrated upward, as clinically tolerated, to identify a maximum stable dose in each subject.

FIG. **13** shows a number of subjects for various maintenance TreT doses in the OEP.

FIG. **14** shows a change in 6 minute walk distance (6MWD) with respect to a baseline 6MWD as a function of duration of TreT treatment.

FIG. **15** is a plot reporting satisfaction of participants of the study of Example 5.

DETAILED DESCRIPTION

It is noted that, as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

As used herein, the term "comprising" or "comprises" is intended to mean that the compositions and methods include the recited elements, but do not exclude others. A composition or method "consisting essentially" of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s) of the claimed technology. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this technology. When an embodiment is defined by one of these terms (e.g., "comprising") it should be understood that this disclosure also includes alternative embodiments, such as "consisting essentially of" and "consisting of" for said embodiment.

"Subject" refers to an animal, such as a mammal (including a human), that has been or will be the object of treatment, observation or experiment. "Subject" and "patient" may be used interchangeably, unless otherwise indicated. The methods described herein may be useful in human therapy and/or veterinary applications. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

The terms "therapeutically effective amount," "effective amount," and "pharmaceutically effective amount" are used interchangeably and refer to an amount of a compound that is sufficient to effect treatment as defined below, when administered to a patient (e.g., a human) in need of such treatment in one or more doses. The therapeutically effective amount will vary depending upon the patient, the disease being treated, the weight and/or age of the patient, the severity of the disease, or the manner of administration as determined by a qualified prescriber or care giver. The therapeutically effective amount can be determined by titrating the dose upwards from a starting dose, either in terms of dose by administration or frequency of administration. In some embodiments, the therapeutically effective dose is determined by titrating the dose upwards until the maximum tolerated dose for the individual subject is determined.

The term "treatment" or "treating" means administering a compound disclosed herein for the purpose of (i) delaying the onset of a disease, that is, causing the clinical symptoms of the disease not to develop or delaying the development thereof, (ii) inhibiting the disease, that is, arresting the

development of clinical symptoms; and/or (iii) relieving the disease, that is, causing the regression of clinical symptoms or the severity thereof.

The term "pulmonary fibrosis" is a condition characterized by scarring and thickening of the lungs. Symptoms include shortness of breath, fatigue, weakness, chronic dry, hacking cough, loss of appetite, and discomfort in the chest. Eventually the scarring in the lung becomes replaced with fibrotic tissue resulting in loss of the lung's ability to transfer oxygen to the blood.

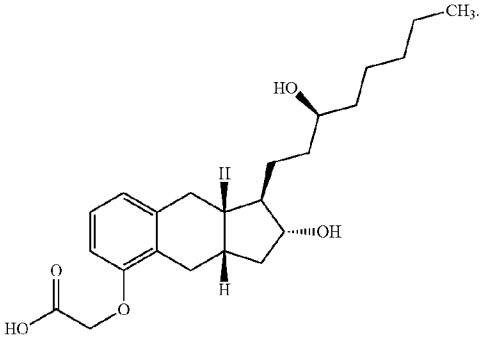
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this present technology belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present technology, representative illustrative methods and materials are described herein.

All numerical designations, e.g., pH, temperature, time, concentration, dose, and molecular weight, including ranges, are approximations which are varied (+) or (−) by increments of 0.05%, 1%, 2%, 5%, 10% or 20%. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about."

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the present technology. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the present technology, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the present technology.

In an aspect, the present disclosure provides a method of treating interstitial lung disease (ILD) in a subject in need, comprising administering to the subject a therapeutically effective amount of treprostinil, a prodrug, salt, or ester thereof.

Treprostinil is used for the treatment of pulmonary arterial hypertension. Treprostinil is a synthetic analog of prostacyclin (PGI_2) having the structure:



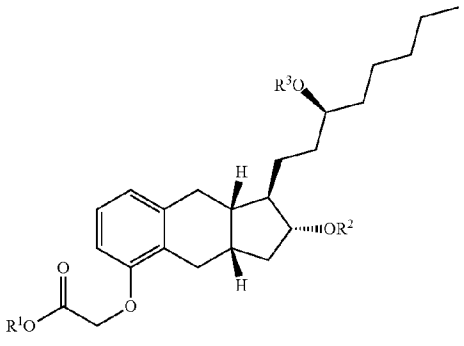
Treprostinil, the active ingredient in Remodulin® (treprostinil) Injection, Tyvaso® (treprostinil) Inhalation Solution, and Orenitram® (treprostinil) Extended Release Tablets, was described in U.S. Pat. No. 4,306,075. Methods of making treprostinil and other prostacyclin derivatives are described, for example, in Moriarty, et al., J. Org. Chem. 2004, 69, 1890-1902, Drug of the Future, 2001, 26(4), 364-374, U.S. Pat. Nos. 6,441,245, 6,528,688, 6,700,025, 6,809,223, 6,756,117, 8,461,393, 8,481,782; 8,242,305, 8,497,393, 8,940,930, 9,029,607, 9,156,786 and 9,388,154 9,346,738; U.S. Published Patent Applications Nos. 2012-0197041, 2013-0331593, 2014-0024856, 2015-0299091, 2015-0376106, 2016-0107973, 2015-0315114, 2016-0152548, and 2016-0175319; PCT Publications No. WO2016/0055819 and WO2016/081658.

Various uses and/or various forms of treprostinil are disclosed, for example, in U.S. Pat. Nos. 5,153,222, 5,234, 953, 6,521,212, 6,756,033, 6,803,386, 7,199,157, 6,054,486, 7,417,070, 7,384,978, 7,879,909, 8,563,614, 8,252,839, 8,536,363, 8,410,169, 8,232,316, 8,609,728, 8,350,079, 8,349,892, 7,999,007, 8,658,694, 8,653,137, 9,029,607, 8,765,813, 9,050,311, 9,199,908, 9,278,901, 8,747,897, 9,358,240, 9,339,507, 9,255,064, 9,278,902, 9,278,903, 9,758,465; 9,422,223; 9,878,972; 9,624,156; U.S. Published Patent Applications Nos. 2009-0036465, 2008-0200449, 2008-0280986, 2009-0124697, 2014-0275616, 2014-0275262, 2013-0184295, 2014-0323567, 2016-0030371, 2016-0051505, 2016-0030355, 2016-0143868, 2015-0328232, 2015-0148414, 2016-0045470, 2016-0129087, 2017-0095432; 2018-0153847 and PCT Publications Nos. WO00/57701, WO20160105538, WO2016038532, WO2018/058124.

A "prodrug" of treprostinil may refer to compounds which are converted in vivo to treprostinil or its pharmaceutically active derivatives thereof, or to a compound described in PCT publication No. WO2005/007081; U.S. Pat. Nos. 7,384,978, 7,417,070, 7,544,713, 8,252,839, 8,410,169, 8,536,363, 9,050,311, 9,199,908, 9,278,901, 9,422,223; 9,624,156, 9,878,972, 9,371,264, 9,394,227, 9,505,737, 9,758,465, 9,643,911, 9,701,616, 9,776,982, 9,845,305, 9,957,200, 10,494,327, 10,053,414, 10,246,403, 10,344, 012, 10,450,290, 10,464,877, 10,464,878, 10,703,706, 10,752,733, 9,255,064, 9,469,600, 10,010,518, 10,343,979, 10,526,274; U.S. Patent Application Publications Nos. 2018-0153847 and 2021-0054009; U.S. provisional patent application No. 63/036,561 filed Jun. 9, 2020; U.S. provisional patent application No. 63/125,145 filed Dec. 14, 2020, each of which is incorporated herein by reference in their entirety.

Prostacyclin is a small molecule that has been previously shown to cause dilation of large blood vessels, relaxation of smooth muscle, inhibition of smooth muscle proliferation, as well as inhibition of platelet aggregation, which is involved in the blood clotting process. Similar actions by treprostinil at the microvascular level and on capillaries near the skin are believed to help enhance cutaneous blood flow and heal and/or prevent ischemia lesions or ulcers associated with scleroderma, Buerger's disease, Raynaud's disease, Raynaud's phenomenon, and other conditions.

An "ester" of treprostinil may refer to a compound of formula:



wherein

R^1 is H, optionally substituted C_1-C_{10} alkyl, optionally substituted C_3-C_{10} cycloalkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_2-C_{10} alkynyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocyclyl;

R^2 and R^3 are each independently —C(O)R^4; and

each R^4 is independently optionally substituted C_1-C_{10} alkyl, optionally substituted C_3-C_{10} cycloalkyl, optionally substituted C_2-C_{10} alkenyl, optionally substituted C_2-C_{10} alkynyl, optionally substituted aryl, optionally substituted heteroaryl, or optionally substituted heterocyclyl;

wherein at least one of R^1, R^2, and R^3, is not H.

"Optionally substituted" refers to a group selected from that group and a substituted form of that group. Substituents may include any of the groups defined below. In one embodiment, substituents are selected from C_1-C_{10} or C_1-C_6 alkyl, substituted C_1-C_{10} or C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_6-C_{10} aryl, C_3-C_5 cycloalkyl, C_2-C_{10} heterocyclyl, C_1-C_{10} heteroaryl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, substituted C_6-C_{10} aryl, substituted C_3-C_8 cycloalkyl, substituted C_2-C_{10} heterocyclyl, substituted C_1-C_{10} heteroaryl, halo, nitro, cyano, —CO_2H or a C_1-C_6 alkyl ester thereof.

"Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and preferably 1 to 6 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH_3—), ethyl (CH_3CH_2—), n-propyl ($CH_3CH_2CH_2$—), isopropyl ((CH_3)$_2$CH—), n-butyl ($CH_3CH_2CH_2CH_2$—), isobutyl ((CH_3)$_2$$CHCH_2$—), sec-butyl (($CH_3$)($CH_3CH_2$)CH—), t-butyl (($CH_3$)$_3$C—), n-pentyl ($CH_3CH_2CH_2CH_2CH_2$), and neopentyl (($CH_3$)$_3$ CCH_2—).

"Alkenyl" refers to monovalent straight or branched hydrocarbyl groups having from 2 to 10 carbon atoms and preferably 2 to 6 carbon atoms or preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of vinyl (>C═C<) unsaturation. Such groups are exemplified, for example, by vinyl, allyl, and but-3-en-1-yl. Included within this term are the cis and trans isomers or mixtures of these isomers.

"Alkynyl" refers to straight or branched monovalent hydrocarbyl groups having from 2 to 10 carbon atoms and preferably 2 to 6 carbon atoms or preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of acetylenic (—C≡C—) unsaturation. Examples of such alkynyl groups include acetylenyl (—C≡CH), and propargyl (—CH_2C≡CH).

"Substituted alkyl" refers to an alkyl group having from 1 to 5, preferably 1 to 3, or more preferably 1 to 2 substituents selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminocarbonyl, aminothiocarbonyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aminosulfonyl, aminosulfonyloxy, aminosulfonylamino, amidino, aryl, substituted aryl, aryloxy, substituted aryloxy, arylthio, substituted arylthio, carboxyl, carboxyl ester, (carboxyl ester)amino, (carboxyl ester)oxy, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, cycloalkylthio, substituted cycloalkylthio, cycloalkenyl, substituted cycloalkenyl, cycloalkenyloxy, substituted cycloalkenyloxy, cycloalkenylthio, substituted cycloalkenylthio, guanidino, substituted guanidino, halo, hydroxy, heteroaryl, substituted heteroaryl, heteroaryloxy, substituted heteroaryloxy, heteroarylthio, substituted heteroarylthio, heterocyclic, substituted heterocyclic, heterocyclyloxy, substituted heterocyclyloxy, heterocyclylthio, substituted heterocyclylthio, nitro, SO_3H, substituted sulfonyl, substituted sulfonyloxy, thioacyl, thiol, alkylthio, and substituted alkylthio, wherein said substituents are as defined herein.

"Substituted alkenyl" refers to alkenyl groups having from 1 to 3 substituents, and preferably 1 to 2 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminocarbonyl, aminothiocarbonyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aminosulfonyl, aminosulfonyloxy, aminosulfonylamino, amidino, aryl, substituted aryl, aryloxy, substituted aryloxy, arylthio, substituted arylthio, carboxyl, carboxyl ester, (carboxyl ester) amino, (carboxyl ester)oxy, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, cycloalkylthio, substituted cycloalkylthio, cycloalkenyl, substituted cycloalkenyl, cycloalkenyloxy, substituted cycloalkenyloxy, cycloalkenylthio, substituted cycloalkenylthio, guanidino, substituted guanidino, halo, hydroxyl, heteroaryl, substituted heteroaryl, heteroaryloxy, substituted heteroaryloxy, heteroarylthio, substituted heteroarylthio, heterocyclic, substituted heterocyclic, heterocyclyloxy, substituted heterocyclyloxy, heterocyclylthio, substituted heterocyclylthio, nitro, SO_3H, substituted sulfonyl, substituted sulfonyloxy, thioacyl, thiol, alkylthio, and substituted alkylthio, wherein said substituents are as defined herein and with the proviso that any hydroxyl or thiol substitution is not attached to a vinyl (unsaturated) carbon atom.

"Substituted alkynyl" refers to alkynyl groups having from 1 to 3 substituents, and preferably 1 to 2 substituents, selected from the group consisting of alkoxy, substituted alkoxy, acyl, acylamino, acyloxy, amino, substituted amino, aminocarbonyl, aminothiocarbonyl, aminocarbonylamino, aminothiocarbonylamino, aminocarbonyloxy, aminosulfonyl, aminosulfonyloxy, aminosulfonylamino, amidino, aryl, substituted aryl, aryloxy, substituted aryloxy, arylthio, substituted arylthio, carboxyl, carboxyl ester, (carboxyl ester) amino, (carboxyl ester)oxy, cyano, cycloalkyl, substituted cycloalkyl, cycloalkyloxy, substituted cycloalkyloxy, cycloalkylthio, substituted cycloalkylthio, cycloalkenyl, substituted cycloalkenyl, cycloalkenyloxy, substituted cycloalkenyloxy, cycloalkenylthio, substituted cycloalkenylthio, guanidino, substituted guanidino, halo, hydroxy, heteroaryl, substituted heteroaryl, heteroaryloxy, substituted heteroaryloxy, heteroarylthio, substituted heteroarylthio, heterocyclic, substituted heterocyclic, heterocyclyloxy, sub-

stituted heterocyclyloxy, heterocyclylthio, substituted heterocyclylthio, nitro, SO_3H, substituted sulfonyl, substituted sulfonyloxy, thioacyl, thiol, alkylthio, and substituted alkylthio, wherein said substituents are as defined herein and with the proviso that any hydroxyl or thiol substitution is not attached to an acetylenic carbon atom.

"Alkoxy" refers to the group 0 alkyl wherein alkyl is defined herein. Alkoxy includes, by way of example, methoxy, ethoxy, n propoxy, isopropoxy, n butoxy, t butoxy, sec butoxy, and n pentoxy.

"Substituted alkoxy" refers to the group 0 (substituted alkyl) wherein substituted alkyl is defined herein.

"Acyl" refers to the groups H—C(O)—, alkyl-C(O)—, substituted alkyl-C(O)—, alkenyl-C(O)—, substituted alkenyl-C(O) , alkynyl-C(O) , substituted alkynyl-C(O) , cycloalkyl-C(O)—, substituted cycloalkyl-C(O)—, cycloalkenyl-C(O)—, substituted cycloalkenyl-C(O)—, aryl-C(O)—, substituted aryl-C(O)—, heteroaryl-C(O)—, substituted heteroaryl-C(O)—, heterocyclic-C(O)—, and substituted heterocyclic-C(O)—, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. Acyl includes the "acetyl" group $CH_3C(O)$—.

"Acylamino" refers to the groups —$NR^{47}C(O)$alkyl, —$NR^{47}C(O)$substituted alkyl, —$NR^{47}C(O)$cycloalkyl, —$NR^{47}C(O)$substituted cycloalkyl, —$NR^{47}C(O)$cycloalkenyl, —$NR^{47}C(O)$substituted cycloalkenyl, —$NR^{47}C(O)$alkenyl, —$NR^{47}C(O)$substituted alkenyl, —$NR^{47}C(O)$alkynyl, —$NR^{47}C(O)$substituted alkynyl, —$NR^{47}C(O)$aryl, $NR^{47}C(O)$substituted aryl, $NR^{47}C(O)$heteroaryl, —$NR^{47}C(O)$substituted heteroaryl, —$NR^{47}C(O)$heterocyclic, and $NR^{47}C(O)$substituted heterocyclic wherein R^{47} is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

"Acyloxy" refers to the groups alkyl-C(O)O , substituted alkyl-C(O)O—, alkenyl-C(O)O—, substituted alkenyl-C(O)O—, alkynyl-C(O)O—, substituted alkynyl-C(O)O—, aryl-C(O)O—, substituted aryl-C(O)O—, cycloalkyl-C(O)O—, substituted cycloalkyl-C(O)O—, cycloalkenyl-C(O)O—, substituted cycloalkenyl-C(O)O—, heteroaryl-C(O)O—, substituted heteroaryl —C(O)O, heterocyclic-C(O)O—, and substituted heterocyclic-C(O)O— wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

"Amino" refers to the group NH_2.

"Substituted amino" refers to the group —$NR^{48}R^{49}$ where R^{48} and R^{49} are independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic, SO_2 alkyl, —SO_2-substituted alkyl, —SO_2-alkenyl, —SO_2-substituted alkenyl, —SO_2-cycloalkyl, —SO_2-substituted cycloalkyl, SO_2-cycloalkenyl, SO_2-substituted cylcoalkenyl, —SO_2-aryl, —SO_2-substituted aryl, —SO_2-heteroaryl, —SO_2-substituted heteroaryl, —SO_2-heterocyclic, and —SO_2-substituted heterocyclic and wherein R^{48} and R^{49} are optionally joined, together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group, provided that R^{48} and R^{49} are both not hydrogen, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. When R^{48} is hydrogen and R^{49} is alkyl, the substituted amino group is sometimes referred to herein as alkylamino. When R^{48} and R^{49} are alkyl, the substituted amino group is sometimes referred to herein as dialkylamino.

When referring to a monosubstituted amino, it is meant that either R^{48} or R^{49} is hydrogen but not both. When referring to a disubstituted amino, it is meant that neither R^{48} nor R^{49} are hydrogen.

"Pharmaceutically acceptable salt" may refer to physiologically acceptable salts of treprostinil, as well as non-physiologically acceptable salts of treprostinil. Pharmaceutically acceptable salts of compounds described herein are within the scope of the present technology and include acid or base addition salts which retain the desired pharmacological activity and is not biologically undesirable (e.g., the salt is not unduly toxic, allergenic, or irritating, and is bioavailable). When the compound of the present technology has a basic group, such as, for example, an amino group, pharmaceutically acceptable salts can be formed with inorganic acids (such as hydrochloric acid, hydroboric acid, nitric acid, sulfuric acid, and phosphoric acid), organic acids (e.g., alginate, formic acid, acetic acid, benzoic acid, gluconic acid, fumaric acid, oxalic acid, tartaric acid, lactic acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, naphthalene sulfonic acid, and p toluenesulfonic acid) or acidic amino acids (such as aspartic acid and glutamic acid). When the compound of the present technology (treprostinil, an ester, prodrug, or derivative thereof) has an acidic group, such as for example, a carboxylic acid group, it can form salts with metals, such as alkali and earth alkali metals (e.g., Na^+, Li^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}), ammonia or organic amines (e.g., dicyclohexylamine, trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, triethanolamine) or basic amino acids (e.g., arginine, lysine and ornithine). Such salts can be prepared in situ during isolation and purification of the compounds or by separately reacting the purified compound in its free base or free acid form with a suitable acid or base, respectively, and isolating the salt thus formed.

ILD may include a range of diseases and disorders, for example, idiopathic pulmonary fibrosis (IPF), desquamative interstitial pneumonia (DIP), acute interstitial pneumonia (AIP), nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis-associated interstitial lung disease (RB-ILD), cryptogenic organizing pneumonia (COP), lymphoid interstitial pneumonia (LIP), sarcoidosis, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, antisynthetase syndrome, silicosis, asbestosis, occupational lung disease, chronic hypersensitivity pneumonitis, idiopathic interstitial pneumonia (IIP), an autoimmune ILD, lymphangioleiomyomatosis (LAM), Langerhans cell histiocytosis (LCH), drug associated ILD, vasculitis, granulomatosis, and berylliosis.

"Pulmonary function" as used herein, refers to the ability of the lungs to absorb oxygen and expand and contract. Pulmonary function, decline thereof, or reduction of the decline, may be assessed using medically recognized tools known to those having ordinary skill in the art. Methods

include pulmonary function testing (PFT), spirometry, lung volumes, maximal respiratory pressure, diffusing capacity, oxygen desaturation, and arterial blood gas evaluation.

"Forced vital capacity" as used herein, refers to the amount of air that can be forcibly exhaled from the lungs after taking the deepest breath possible, as measured by spirometry.

Further aspects of the present invention are concerned with the use of treprostinil or its derivatives, prodrugs, esters, or pharmaceutically acceptable salts thereof, in the manufacture of a medicament for the treatment or prevention of interstitial lung disease or a condition associated with interstitial lung disease. In some embodiments, the medicament is formulated for inhalation. When administered by inhalation, the formulation can be nebulized or formulated for a dry powder inhaler (DPI).

The amount of treprostinil or its derivative, or a pharmaceutically acceptable salt thereof, that is required in methods may depend on a number of factors, such as the specific indication it is being used for, the nature of the particular compound used, the mode of administration, the concentration, and the weight and condition of the subject. A daily dose per subject for ILD, or conditions associated with ILD may be in the range 25 μg to 250 mg or 7 μg to 285 μg, per day per kilogram bodyweight. In some embodiments, the daily dose may be in the range of about 150 μg to about 350 μg per day, about 200 μg to about 300 μg per day, or about 225 μg to about 275 μg per day. Intravenous doses in the range 0.5 μg to 1.5 mg per kilogram bodyweight per day may be administered as an infusion of from 0.5 ng to 1.0 μg per kilogram bodyweight per minute.

The treprostinil or its derivative, prodrug, ester, or a pharmaceutically acceptable salt thereof, can be administered using any suitable treatment schedule. In some embodiments, the drug will be administered multiple times a day (1, 2, 3, 4, or 5), and in other embodiments, the drug can be continuously administered, such as by using an infusion pump. The duration of treatment can vary depending on the severity of disease, treatment goals, or individual circumstances. In some embodiments, the duration of treatment is at least one week, at least two weeks, at least four weeks, at least eight weeks, or at least sixteen weeks. In some embodiments, the duration of treatment is indefinite, e.g., treatment can continue for the life of the subject or until disease symptoms decrease below some threshold.

Pharmaceutical compositions described herein or administered to subjects, hereinafter referred to as a "formulation" or "composition," of treprostinil and/or its prodrugs, esters, derivatives, and/or pharmaceutically acceptable salts thereof, may be admixed with, inter alia, an acceptable carrier. The carrier may be compatible with any other ingredients in the formulation and not deleterious to the subject. The carrier may be a solid or a liquid, or both. One or more of treprostinil or its derivatives, esters, prodrugs, or pharmaceutically acceptable salts thereof, may be incorporated in the formulations of the invention. Formulations administered include those suitable for parenteral, oral, inhalation, rectal, topical, buccal and transdermal administration.

Parenterally administered compositions may be isotonic with the blood of the intended recipient. Subcutaneous injection, intravenous, intramuscular or intradermal injection may be used. Such preparations may conveniently be prepared by admixing the compound with water or a glycine or citrate buffer and rendering the resulting solution sterile and isotonic with the blood.

Formulations suitable for oral administration may be presented as capsules, cachets, lozenges, or tablets, each containing a specific amount of treprostinil or its derivative, prodrug, ester, or a pharmaceutically acceptable salt thereof; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion. Oral formulations that may be administered include those described in U.S. Pat. Nos. 7,384,978 and 8,747,897 (including the commercial product Orenitram® (treprostinil) Extended-Release Tablets), the entire disclosures of which are hereby incorporated by reference. In general, the formulations of the invention are prepared by uniformly and intimately admixing treprostinil, an ester, prodrug, or salt thereof with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the resulting mixture.

Formulations suitable for buccal (sub-lingual) administration include lozenges comprising treprostinil or its derivative, prodrug, ester, or a pharmaceutically acceptable salt thereof, in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

Formulations suitable for rectal administration are preferably presented as unit dose suppositories. These may be prepared by admixing treprostinil or its derivative, prodrug, ester, or a pharmaceutically acceptable salt thereof, with one or more solid carriers.

Topical and transdermal formulations me be an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers possible include vaseline, lanoline, polyethylene glycols, alcohols, and combinations thereof.

Treprostinil, prodrugs, esters, and salts thereof are conveniently prepared by methods the same as or analogous to those described in U.S. Pat. Nos. 4,306,075, 6,528,688 and 6,441,245, the disclosures of which are hereby incorporated by reference.

In some embodiments of the present methods, the treprostinil administered is provided as a kit with instructions for use in treating ILD. In certain kit embodiments, the treprostinil or its derivative, prodrug, ester, or a pharmaceutically acceptable salt thereof, is in a form suitable for subcutaneous administration, continuous subcutaneous infusion, intravenously administration or inhalation. Subcutaneous formulations administered to the subject may include any of those described in U.S. Pat. No. 7,999,007 (including the commercial product Remodulin® (treprostinil) Injection), the entire disclosure of which is hereby incorporated by reference. In other kit embodiments, the treprostinil or its derivative, or a pharmaceutically acceptable salt thereof, is in an orally available form selected from the group consisting of tablets and capsules.

The effects of the method on pulmonary fibroses (PF) can be ascertained via an animal model of PF such as bleomycin and vanadium pentoxide (V205) models as described in Bonner J C, Rice A B, Ingram J L, Moomaw C R, Nyska A, Bradbury A, Sessoms A R, Chulada P C, Morgan D L, Zeldin D C, and Langenbach R. Susceptibility of cyclooxygenase-2-deficient mice to pulmonary fibrogenesis. *Am J Pathol* 161: 459-470, 2002; 23; and Keerthisingam C B, Jenkins R G, Harrison N K, Hernandez-Rodriguez N A, Booth H, Laurent G J, Hart S L, Foster M L, and McAnulty R J. Cyclooxygenase-2 deficiency results in a loss of the anti-proliferative response to transforming growth 31 factor-beta in human fibrotic lung fibroblasts and promotes bleomycin-induced pulmonary fibrosis in mice. *Am J Pathol* 158: 1411-1422, 2001, incorporated herein by reference in their entirety.

In preferred embodiments, treprostinil is administered via inhalation. Inhaled compositions comprising treprostinil may include sprays, aerosols, and dry powder compositions. Said compositions may include a variety of excipients. Inhalable compositions administered may include any of those described in U.S. Pat. No. 9,339,507 (including the commercial product Tyvaso® (treprostinil) Inhalation Solution), WO2017192993 and WO2014085813, the entire disclosures of which are hereby incorporated by reference.

The excipient or excipients of the pharmaceutical composition according to the invention may have water solubility greater than 5 g/l and often greater than 100 g/l and more. They are preferably chosen among sugars, salts or amino acids and have double function of minimizing the effect of the inhaled composition on the fluid's cellular outcome. Regarding the composition in its solid dry form, the excipient also forms the solid matrix in which the treprostinil, a prodrug, ester, salt, or derivative thereof is dispersed.

The composition may include excipients such as lactose, corn starch, or the like, glidants such as magnesium stearate, etc., emulsifying agents, suspending agents, stabilizers, and isotonic agents, etc. If desired, a sweetening agent and/or a flavoring agent may be added. Exemplary excipients include, without limitation, polyethylene glycol (PEG), hydrogenated castor oil (HCO), cremophors, carbohydrates, starches (e.g., corn starch), inorganic salts, antimicrobial agents, antioxidants, binders/fillers, surfactants, lubricants (e.g., calcium or magnesium stearate), glidants such as talc, disintegrants, diluents, buffers, acids, bases, film coats, combinations thereof, and the like. Other examples of soluble excipients that may be used in the composition according to the invention are alitame, acesulfame potassium, aspartame, saccharin, sodium saccharin, sodium cyclamate, sucralose, threalose, xylitol, citric acid, tartaric acid, cyclodextrins, dextrins, hydroxyethylcellulose, gelatine, malic acid, maltitol, maltodextrin, maltose, polydextrose, tartaric acid, sodium or potassium bicarbonate, sodium or potassium chloride, sodium or potassium citrate, phospholipids, lactose, sucrose, glucose, fructose, mannitol, sorbitol, natural aminoacids, alanine, glycine, serine, cysteine, phenylalanine, tyrosine, tryptophan, histidine, methionine, threonine, valine, isoleucine, leucine, arginine, lysine, aspartic acid, glutamic acid, asparagine, glutamine, proline, their salts, and their possible simple chemical modifications such as in N-acetylcysteine, and carbocysteine.

The preferred soluble excipients are alkaline metals salts such as sodium chloride or potassium chloride, and sugars, such as lactose. Specific carbohydrate excipients include, for example, monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, and the like.

As far as the hollow morphology of the particles of the dry powder is concerned, the composition requires the presence of a soluble excipient, preferably a sugar like lactose, able to form in the beginning of the solvent evaporation phase during preparation of the composition, during spray-drying, the backbone of the particle, producing high porosity particles.

In some embodiments, the excipient comprises a surfactant. The surfactant of the composition can be chosen among different classes of surfactants of pharmaceutical use.

Surfactants suitable to be used in the present invention are all those substances characterized by medium or low molecular weight that contain a hydrophobic moiety, generally readily soluble in an organic solvent but weakly soluble or insoluble in water, and a hydrophilic (or polar) moiety, weakly soluble or insoluble in an organic solvent but readily soluble in water. Surfactants are classified according to their polar moiety. Therefore, surfactant with a negatively charged polar moiety are called anionic surfactants, while cationic surfactants have a positively charged polar moiety. Uncharged surfactant are generally called non-ionic, while surfactant charged both positively and negatively are called zwitterionic. Examples of anionic surfactants are salts of fatty acids (better known as soaps), sulfates, sulfate ethers and phosphate esters. Cationic surfactants are frequently based on polar groups containing amino groups. Most common non-ionic surfactants are based on polar groups containing oligo-(ethylene-oxide) groups. Zwitterionic surfactants are generally characterized by a polar group formed by a quaternary amine and a sulfuric or carboxylic group.

Specific examples of this application are the following surfactants: benzalkonium chloride, cetrimide, docusate sodium, glyceryl monolaurate, sorbitan esters, sodium lauryl sulfate, polysorbates, phospholipids, biliary salts.

Non-ionic surfactants, such as polysorbates and polyethylene and polyoxypropylene block copolymers, known as "Poloxamers," may be used. Polysorbates are described in the CTFA International Cosmetic Ingredient Dictionary as mixtures of sorbitol and sorbitol anhydride fatty acid esters condensed with ethylene oxide. Particularly preferred are non-ionic surfactants of the series known as "Tween," in particular the surfactant known as "Tween 80," a polyoxyethylensorbitan. Additional exemplary excipients include surfactants such as other polysorbates, e.g., "Tween 20" and pluronics such as F68 and F88 (both of which are available from BASF, Mount Olive, N.J.), sorbitan esters, lipids (e.g., phospholipids such as lecithin and other phosphatidylcholines, and phosphatidylethanolamines), fatty acids and fatty esters, steroids such as cholesterol, and chelating agents, such as EDTA, zinc and other such suitable cations.

The presence of a surfactant, and preferably of Tween 80, may be necessary to reduce electrostatic charges found in compositions without it, the flow of the powder and the maintenance of the solid state in a homogeneous way without initial crystallization. According to the present invention, phospholipids are included in the above-mentioned definition of surfactants or excipients.

The inhalatory formulation according administered can include a hydrophobic substance in order to reduce sensitivity to humidity. Such hydrophobic substance is preferably leucine, which makes the particle disaggregation easier.

In case of production of a solid product in powder form, this can occur using different techniques, well consolidated in the pharmaceutical industry. The preparation of fine particles through spray-drying represents a preferred method according to the invention. In case of industrial production, this technique is undoubtedly preferred to freeze-drying, which at the moment is the most expensive drying process, both for the apparatus used, and for the yield and production times.

The pharmaceutical composition according to the invention can include other components, such as pH buffers and preservatives. Buffers include, but are not limited to, citric acid, sodium chloride, potassium chloride, sodium sulfate, potassium nitrate, sodium phosphate monobasic, sodium phosphate dibasic, and combinations thereof.

Further, a composition administered may optionally include one or more acids or bases. Non-limiting examples of acids that can be used include those acids selected from

the group consisting of hydrochloric acid, acetic acid, phosphoric acid, citric acid, malic acid, lactic acid, formic acid, trichloroacetic acid, nitric acid, perchloric acid, phosphoric acid, sulfuric acid, fumaric acid, and combinations thereof. Non-limiting examples of suitable bases include bases selected from the group consisting of sodium hydroxide, sodium acetate, ammonium hydroxide, potassium hydroxide, ammonium acetate, potassium acetate, sodium phosphate, potassium phosphate, sodium citrate, sodium formate, sodium sulfate, potassium sulfate, potassium fumerate, and combinations thereof.

The excipients may include an antioxidant, for example, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, and combinations thereof.

The term "dry powder" in reference to the composition of the invention, refers to a powder, granulate, tablet form composition, or any other solid form with a humidity content that assures to the composition chemical stability in time. More precisely, the term "dry" refers to a solid composition with water content lower than 10% w/w, normally less than 5% and preferably less than 3%.

The amount of any excipient in the dry powder composition of the invention can change within a wide range. The amount of any individual excipient in the composition will vary depending on the role of the excipient, the dosage requirements of the active agent components, and particular needs of the composition. Generally, however, the excipient will be present in the composition in an amount of about 1% to about 99% by weight, preferably from about 5% to about 98% by weight, more preferably from about 15% to about 95% by weight of the excipient. In general, the amount of excipient present in a composition of the disclosure is selected from the following: at least about 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or even 95% by weight.

The treprostinil composition administered may be provided as a kit that includes a metered dose inhaler containing a pharmaceutical composition comprising treprostinil or its derivative, ester, prodrug, or a pharmaceutically acceptable salt thereof. Such a kit can further include instructions on how to use the metered dose inhaler for inhaling treprostinil. Such instructions can include, for example, information on how to coordinate patient's breathing, and actuation of the inhaler. The kit can be used by a subject, such as human being, affected with ILD that can be treated by treprostinil. In some cases, the kit is a kit for treating ILD, that includes (i) a metered dose inhaler containing a pharmaceutical composition comprising treprostinil or its derivative, ester, prodrug, or a pharmaceutically acceptable salt thereof; and (ii) instructions for use of the metered dose inhaler containing treprostinil in treating pulmonary hypertension.

The present disclosure also provides a method of treating a pulmonary hypertension due to a condition selected from a chronic lung disease and/or hypoxia (low oxygen levels) by administering to a subject, such as a human being, with such the pulmonary hypertension an effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug. Pulmonary hypertension due to a chronic lung disease and/or hypoxia belongs Group 3 pulmonary hypertension according to the World Health Organization (WHO) classification.

The chronic lung disease may include an obstructive lung disease in which the lung airways are narrow and make it difficult to exhale, such as chronic obstructive pulmonary disease (COPD) and emphysema; a restrictive lung disease in which the lungs have a difficult time expanding when one inhales, such as interstitial lung disease or pulmonary fibrosis; sleep apnea; living in an area of high altitude for a long period of time; and various combinations of the above conditions.

In some embodiments, the chronic lung disease may include idiopathic interstitial pneumonia, such as idiopathic pulmonary fibrosis, idiopathic nonspecific interstitial pneumonia, respiratory bronchiolitis (e.g. respiratory bronchiolitis associated with interstitial lung disease), desquamative interstitial pneumonia, acute interstitial pneumonia; chronic hypersensitivity pneumonitis, occupational lung disease, pulmonary fibrosis, emphysema, connective tissue disease or any combination of the above conditions.

In some embodiments, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may provide an increase, which may be statistically significant, in a six minute walk distance (6MWD) in a subject with a pulmonary hypertension due to a condition selected from a chronic lung disease and/or hypoxia compared to a baseline 6MWD value, i.e. a 6MWD value prior to the administering. For example, the 6MWD value may be statistically significantly increased after at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks of the administering. In some embodiments, the administering may provide an increase of at least 5 m, at least 10 m or at least 15 m in the 6MWD compared to the baseline 6MWD value after at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks of the administering. In some embodiments, the administering may provide an increase of at least 5 m, at least 10 m, at least 15 m, at least 18 m or at least 20 m in the 6MWD compared to the baseline 6MWD value after at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks of the administering.

In some embodiments, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may provide an reduction, which may be statistically significant, in a plasma concentration of NT-proBNP in a subject with a pulmonary hypertension due to a condition selected from a chronic lung disease and/or hypoxia compared to a baseline NT-proBNP plasma concentration, i.e. a NT-proBNP plasma concentration value prior to the administering. For example, the NT-proBNP plasma concentration may be statistically significantly reduced after at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks of the administering. In some embodiments, the administering may provide a reduction of at least 50 pg/ml, at least 100 pg/ml, at least 150 pg/ml, at least 200 pg/ml, at least 250 pg/ml, at least 300 pg/ml or at least 350 pg/ml in the NT-proBNP plasma concentration compared to

the baseline the NT-proBNP plasma concentration value after at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks of the administering.

In some embodiments, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug to a subject with a pulmonary hypertension due to a chronic lung disease may provide a reduction, which may be statistically significant, of a number of exacerbation(s) of the chronic lung disease. For example, a number of exacerbation(s) of the chronic lung disease may be lower in a patient subpopulation with the pulmonary hypertension due to the chronic lung disease, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug for at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks, compared to a patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. For example, the number of exacerbation(s) may be lowered by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80%. The exacerbation(s) may include an acute, clinically significant, respiratory deterioration characterized by evidence of new widespread alveolar abnormality.

In some embodiments, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug to a subject with a pulmonary hypertension due to a chronic lung disease and/or hypoxia may provide a reduction, which may be statistically significant, of a number of clinical worsening event(s). For example, a number of clinical worsening event(s) may be lower in a patient subpopulation with the pulmonary hypertension due to the chronic lung disease and/or hypoxia, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug for at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks compared to a patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. For example, the number of clinical worsening event(s) may be lowered by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70% or at least 80%. The clinical worsening event(s) may include one or more of hospitalization due to a cardiopulmonary indication, a decrease in a 6MWD by more than 15% from a baseline 6MWD value, death or a lung transplantation.

In some embodiments, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may provide an improvement, which may be statistically significant, in forced vital capacity (FVC) in a subject with a pulmonary hypertension due to a condition selected from a chronic lung disease and/or hypoxia. For example, the FVC may be higher in a patient subpopulation with the pulmonary hypertension due to the chronic lung disease and/or hypoxia, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug for at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks, or at least 20 weeks or at least 24 weeks or at least 28 weeks or at least 32 weeks or at least 36 weeks or at least 40 weeks or at least 44 weeks or at least 48 weeks or at least 52 weeks, compared to a patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. For example, the FVC value may be higher by at least 10 ml or at least 15 ml or at least 20 ml or at least 25 ml or at least 30 ml or at least 35 ml or at least 40 ml or at least 45 ml after at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks, at least 11 weeks, at least 12 weeks, at least 13 weeks, at least 14 weeks, at least 15 weeks or at least 16 weeks of the administering in the patient subpopulation with the pulmonary hypertension due to the chronic lung disease and/or hypoxia, who was administered the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug compared to the patient subpopulation with the same condition, which was administered a placebo instead of treprostinil. In patients with a chronic lung disease, such as interstitial lung disease, and/or hypoxia, an FVC value usually decreases with time when untreated. Thus, administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt may increase an FVC value compared to an FVC value before the administering; maintain an FVC value within 5%, 10% or 20% within the FVC value prior to the administering; or reduce a decrease of an FVC value with time compared to a decrease in an FVC value with no administering the effective amount of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt, such a decrease in an FVC value when placebo is administered instead of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt.

In some embodiments, treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may be administered by inhalation, which may be, for example, an oral inhalation or a nasal inhalation. In some embodiments, treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may be administered by an inhalation device, which may be for example, a pulsed inhalation device, such as a metered dose inhaler and/or a pulsed nebulizer. Pulsed inhalation devices are disclosed, for example, in U.S. patent application publication No. 20080200449, U.S. Pat. Nos. 9,358,240; 9,339,507; 10,376,525; and 10,716,793, each of which is incorporated herein by reference in its entirety.

In some embodiments, the inhalation device, such as a pulsed inhalation device, may contain a solution or a suspension comprising treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug. For example, such solution or suspension may be used for aerosolizing or a nebulizing by an inhalation device, such as a nebulizer and/or a metered dose inhaler. One example of a solution may be a commercial product Tyvaso®. A concentration of treprostinil in such solution may vary. In some embodiments, the treprostinil concentra-

tion may be from 200 μg/ml to 2000 μg/ml or from 300 μg/ml to 1500 μg/ml or from 400 μg/ml to 1200 μg/ml or any value or subrange within these ranges. For example, in a certain embodiment, the treprostinil concentration may be 600 μg/ml.

In some embodiments, the inhalation device, such as a pulsed inhalation device, may be a dry powder inhaler, which may contain a dry powder composition or formulation comprising treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug. For example, a dry powder inhaler and a dry powder composition or formulation comprising treprostinil are disclosed in WO2019/237028, which incorporated herein by reference in its entirety. In some embodiments, in addition to treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug, the dry powder composition may further a diketopiperazine, such as (E)-3,6-bis[4-(N-carbonyl-2-propenyl) amidobutyl]-2,5-diketopiperazine (FDKP).

Treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug may be administered by inhalation in a single administering event which may involve a limited number of breaths (or inhalations) by the subject. For example, in some embodiments, a number of breaths in the single administering event may not exceed 20 breaths (or inhalations) or 19 breaths (or inhalations) or 18 breaths (or inhalations) or 17 breaths (or inhalations) or 16 breaths (or inhalations) or 15 breaths (or inhalations) or 14 breaths (or inhalations) or 13 breaths (or inhalations) or 12 breaths (or inhalations) or 11 breaths (or inhalations) or 10 breaths (or inhalations) or 9 breaths (or breaths (or inhalations) inhalations) or 8 breaths (or inhalations) or 7 breaths (or inhalations) or 6 breaths (or inhalations) or 5 breaths (or inhalations) or 4 breaths (or inhalations) or 3 breaths (or inhalations) or 2 breaths (or inhalations) or 1 breath (or inhalation).

A dose of treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug administered by inhalation in a single administering event may vary. In some embodiments, the single administering event dose may be from 7.5 μg to 100 μg or 10 μg to 100 μg or 15 μg to 100 μg from 15 μg to 90 μg or from 15 μg to 75 μg or from 30 μg to 75 μg or any value or subrange within these ranges.

A number of single administering events per day for administering treprostinil, its prodrug, its pharmaceutically acceptable salt or a pharmaceutically acceptable salt of its prodrug administered by inhalation may vary. For example, the number of single administering events per day may be 1, 2, 3, 4, 5 or 6 per day.

The table below provides exemplary doses of treprostinil in a dry powder formulation, which may be used in a dry powder inhaler, and how they may compare with treprostinil doses in Tyvaso® inhalation solution.

DPI (treprostinil) Inhalation Powder Cartridge Strength (QID)	Tyvaso (treprostinil) Inhalation Solution Number of Breaths (QID)
16 mcg	2 to 4 (18 to 24 mcg)
32 mcg	5 to 7 (30 to 42 mcg)
48 mcg	8 to 10 (48 to 60 mcg)
64 mcg	11 to 13 (66 to 78 mcg)

The disclosure of all publications cited above are expressly incorporated herein by reference in their entireties to the same extent as if each were incorporated by reference individually.

The examples described herein are illustrative of the present invention and are not intended to be limitations thereon. Different embodiments of the present invention have been described according to the present invention. Many modifications and variations may be made to the techniques described and illustrated herein without departing from the spirit and scope of the invention. Accordingly, it should be understood that the examples are illustrative only and are not limiting upon the scope of the invention.

EXAMPLES

Example 1: Inhaled Treprostinil Results on Underlying Lung Disease

An exacerbation of underlying lung disease is defined as an acute, clinically significant, respiratory deterioration characterized by evidence of new widespread alveolar abnormality (Collard et al., 2016). The present example shows that treatment with inhaled treprostinil resulted in significantly fewer exacerbations of underlying lung disease in patients.

Subjects having underlying lung disease were treated with inhaled treprostinil over 16 weeks. Subjects initiated inhaled treprostinil or placebo at a dose of 3 breaths (18 mcg) 4 times daily (during waking hours). Study drug doses were maximized throughout the study. Dose escalations (additional 1 breath 4 times daily) could occur up to every 3 days with a target dosing regimen of 9 breaths (54 mcg) 4 times daily and a maximum dose of 12 breaths (72 mcg) 4 times daily, as clinically tolerated. Subjects were assessed during Screening and Baseline to determine eligibility for the study. Once eligible, 5 Treatment Phase visits to the clinic were required at Week 4, Week 8, Week 12, Week 15, and Week 16 (final study visit). An Early Termination (ET) Visit was conducted for subjects who discontinued prior to Week 16; all assessments planned for the final Week 16 Visit were conducted during the ET Visit, if applicable. Subjects were contacted at least weekly by telephone or email to assess tolerance to study drug, adverse events (AEs), and changes to concomitant medications.

Efficacy assessments consisted of 6MWD, plasma NT-proBNP concentration, and time to clinical worsening. Exploratory endpoints included SGRQ, change in DSP, time to exacerbation of underlying disease, and pulmonary function tests. Safety assessments consisted of the development of AEs, vital signs, clinical laboratory parameters, ECG parameters, hospitalizations due to cardiopulmonary indications, exacerbations of underlying lung disease, and oxygenation.

Treatment resulted in significantly fewer exacerbations of underlying lung disease over the 16-week treatment period (26.4% in Inhaled Treprostinil group and 38.7% in placebo group; p=0.018) and decreased risk of an exacerbation of underlying lung disease (hazard ratio 0.66 or 34% reduction in risk) as shown in FIG. 1.

In addition, the following FVC suggestive data was obtained from this study. Among patients treated with inhaled treprostinil, overall results from intent to treat group were:

Overall ITT
- 28.47 mL and 44.40 mL in FVC at Weeks 8 and 16
- Percent predicted FVC at Week 8 (1.79%; p–0.0139) and Week 16 (1.80%; p=0.0277).
- Subset IIP etiology:
  - 46.48 mL and 108.18 mL (p=0.0229) at Weeks 8 and 16
  - Percent predicted FVC at Week 8 (1.95%, p=0.0373) and Week 16 (2.88%; p=0.0096)
- Subset IPF etiology:
  - 84.52 mL and 168.52 mL (p=0.0108) at Weeks 8 and 16
  - Percent predicted FVC at Week 8 (2.54%; p=0.0380) and Week 16 (3.50%; p=0.0147)

Nintedanib: IPF~109 mL (3.2% predicted) at 52 weeks
Pirfenidone: IPF~153-193 mL at 52 weeks

Placebo corrected, rate of decline (not improvements)

In comparison to the known treatments for ILD (nintedanib and pirfenidone) shown above, inhaled treprostinil achieves comparable effects with shorter treatment duration.

Pulmonary function testing was initially conducted as a safety assessment (Safety Population) during the study. The results indicated that although most PFT parameters remained stable for subjects in the study, a notable improvement in FVC (% predicted) was observed at Week 16 in the inhaled treprostinil group (median improvement of 1.0% compared to a 1.0% reduction in the placebo group). As a result, post hoc MMRM analyses of FVC data were performed for the ITT Population and are presented in Table 1 (ITT Population), Table 2 (by PH ILD Etiology of IIP) and Table 3 (for subjects with IPF), shown below.

TABLE 1

Analysis of FVC Data Using Mixed Model Repeated Measurement - ITT Population

Visit	Treatment	N	LS Mean	Contrast	Estimated Difference	95% CI	p-value
			FVC (mL)				
Week 8	Inhaled treprostinil	142	5.49	Inhaled treprostinil – Placebo	28.47	−30.81, 87.74	0.3453
	Placebo	141	−22.98				
Week 16	Inhaled treprostinil	130	9.77	Inhaled treprostinil – Placebo	44.40	−25.25, 114.05	0.2106
	Placebo	126	−34.63				
			FVC (% predicted)				
Week 8	Inhaled treprostinil	142	0.77	Inhaled treprostinil – Placebo	1.79	0.37, 3.21	0.0139
	Placebo	141	−1.02				
Week 16	Inhaled treprostinil	130	1.07	Inhaled treprostinil – Placebo	1.80	0.20, 3.39	0.0277
	Placebo	126	−0.72				

Abbreviations:

CI, confidence interval;

FVC, forced vital capacity;

ITT, Intent-to-Treat;

LS, least square;

MMRM, mixed model repeated measurement

LSMean, p-values, estimated difference, and associated 95% CI were from the MMRM with the change from baseline in FVC/% predicted FVC as the dependent variable; treatment, week, treatment by week interaction as the fixed effects; baseline FVC/% predicted FVC as the covariate; and subject as the random effect. An unstructured variance/covariance structure shared across treatment groups was used to model the within-subject errors.

TABLE 2

Analysis of FVC Data Using Mixed Model Repeated Measurement for PH-ILD Etiology of IIP - ITT Population

Visit	Treatment	N	LS Mean	Contrast	Estimated Difference	95% CI	p-value
			PH-ILD Etiology: IIP FVC (mL)				
Week 8	Inhaled treprostinil	58	9.27	Inhaled treprostinil – Placebo	46.48	−32.55, 125.51	0.2467
	Placebo	71	−37.21				
Week 16	Inhaled treprostinil	52	22.16	Inhaled treprostinil – Placebo	108.18	15.25, 201.10	0.0229
	Placebo	63	−86.02				
			FVC (% predicted)				
Week 8	Inhaled treprostinil	58	0.92	Inhaled treprostinil – Placebo	1.95	0.12, 3.79	0.0373
	Placebo	71	−1.03				
Week 16	Inhaled treprostinil	52	1.66	Inhaled treprostinil – Placebo	2.88	0.72, 5.05	0.0096
	Placebo	63	−1.23				

Abbreviations:

CI, confidence interval;

CPFE, combined pulmonary fibrosis and emphysema;

CTD, connective tissue disease;

FVC, forced vital capacity;

ILD, interstitial lung disease;

IIP, idiopathic interstitial pneumonia;

ITT, Intent-to-Treat;

LS, least square;

MMRM, mixed model repeated measurement

LSMean, p-values, estimated difference, and associated 95% CI were from the MMRM with the change from baseline in FVC/% predicted FVC as the dependent variable; treatment, week, treatment by week interaction as the fixed effects; baseline FVC/% predicted FVC as the covariate; and subject as the random effect. An unstructured variance/covariance structure shared across treatment groups was used to model the within-subject errors.

Table 3: Analysis of FVC Data Using Mixed Model Repeated Measurement for Subjects with IPF - ITT for IIP Subjects

			IPF				
			FVC (mL)				
Week 8	Inhaled treprostinil	31	41.69	Inhaled treprostinil - Placebo	84.522	−20.409, 189.454	0.1128
	Placebo	47	−42.83				
Week 16	Inhaled treprostinil	28	38.24	Inhaled treprostinil - Placebo	168.524	40.078, 296.970	0.0108
	Placebo	42	−130.3				
			FVC (% predicted)				
Week 8	Inhaled treprostinil	31	1.60	Inhaled treprostinil - Placebo	2.543	0.145, 4.941	0.0380
	Placebo	47	−0.94				
Week 16	Inhaled treprostinil	28	1.62	Inhaled treprostinil - Placebo	3.504	0.712, 6.295	0.0147
	Placebo	42	−1.88				

Abbreviations:

CI, confidence interval;

FVC, forced vital capacity;

IPF, idiopathic pulmonary fibrosis;

ITT, Intent-to-Treat;

LS, least square; MMRM, mixed model repeated measurement

LSMean, p-values, estimated difference, and associated 95% CI were from the MMRM with the change from baseline in FVC/% predicted FVC as the dependent variable; treatment, week, treatment by week interaction as the fixed effects; baseline FVC/% predicted FVC as the covariate; and subject as the random effect. An unstructured variance/covariance structure shared across treatment groups was used to model the within-subject errors.

Treatment with inhaled treprostinil resulted in improvements of 28.47 mL and 44.40 mL in FVC at Weeks 8 and 16, respectively; significant when presented as % predicted FVC at Week 8 (1.79%; p=0.0139) and Week 16 (1.80%; p=0.0277).

When FVC was analyzed by PH-ILD etiology of IIP, treatment with inhaled treprostinil resulted in improvements of 46.48 mL and 108.18 mL (p−0.0229) when compared to placebo at Weeks 8 and 16, respectively. The between group differences for % predicted FVC were statistically significant at Week 8 (1.95%, p=0.0373) and Week 16 (2.88%; p=0.0096).

Further analysis of FVC for subjects with an IPF etiology (using only the IIP subjects in the ITT Population), showed that treatment with inhaled treprostinil resulted in improvements of 84.52 mL and 168.52 mL (p=0.0108) compared to placebo at Weeks 8 and 16, respectively. The between group differences for % predicted FVC were statistically significant at Week 8 (2.54%; p=0.0380) and Week 16 (3.50%; p=0.0147).

Example 2

The following prophetic example will assess efficacy of treprostinil as indicated for the treatment of chronic fibrosing interstitial lung diseases (CF-ILDs) including Idiopathic Interstitial Pneumonias (IIPs) including IPF, chronic hypersensitivity pneumonitis (CHP), and environmental/occupational fibrosing lung disease.

Patients may be treated with inhaled treprostinil up to 15 breaths QID based upon tolerability. Change from baseline to Week 24 of treatment in FVC (absolute or percent predicted) as primary efficacy endpoint will be assessed. Parameters that may be assessed include time to exacerbation of underlying lung disease, 6 meter walk distance test (6MWD), all-cause mortality/survival, time to death, additional analyses of FVC (e.g. absolute and relative change), changes from baseline in SpO_2, diffusing capacity of the lungs for carbon monoxide (DLCO), NT-proBNP, and King's Brief Interstitial Lung Disease Questionnaire.

REFERENCES

1. Collard et al., *American Journal of Respiratory and Critical Care Medicine*, Volume 194 Number 3, pg. 265.

2. Meyer et al., (Apr. 3, 2017). *Therapeutics and Clinical Risk Management*. 13: 427-437.

Example 3: Inhaled Treprostinil in Pulmonary Hypertension Due to Interstitial Lung Disease

No therapies are currently approved for the treatment of pulmonary hypertension in patients with interstitial lung disease. The safety and efficacy of inhaled treprostinil for patients with this condition are unclear.

Methods

We enrolled patients with interstitial lung disease and pulmonary hypertension (documented by right heart catheterization) in a multicenter, randomized, double-blind, placebo-controlled, 16-week trial. Patients were assigned in a 1:1 ratio to receive inhaled treprostinil, administered by means of an ultrasonic, pulsed-delivery nebulizer in up to 12 breaths (total, 72 μg) four times daily, or placebo. The primary efficacy end point was the difference between the two treatment groups in the change in peak 6-minute walk distance from baseline to week 16. Secondary end points included the change in N-terminal pro-B-type natriuretic peptide (NT-proBNP) level at week 16 and the time to clinical worsening.

Results

A total of 326 patients underwent randomization, with 163 assigned to inhaled treprostinil and 163 to placebo. Baseline characteristics were similar in the two groups. At week 16, the least-squares mean difference between the treprostinil group and the placebo group in the change from baseline in the 6-minute walk distance was 31.12 m (95% confidence interval [CI]. 16.85 to 45.39; P<0.001). There was a reduction of 15% in NT-proBNP levels from baseline with inhaled treprostinil as compared with an increase of 46% with placebo (treatment ratio, 0.58; 95% CI, 0.47 to 0.72; P<0.001). Clinical worsening occurred in 37 patients (22.7%) in the treprostinil group as compared with 54 patients (33.1%) in the placebo group (hazard ratio, 0.61;

95% CI, 0.40 to 0.92; P=0.04 by the log-rank test). The most frequently reported adverse events were cough, headache, dyspnea, dizziness, nausea, fatigue, and diarrhea.

Conclusions

In patients with pulmonary hypertension due to interstitial lung disease, inhaled treprostinil improved exercise capacity from baseline, assessed with the use of a 6-minute walk test, as compared with placebo.

Precapillary pulmonary hypertension is defined as an elevation in mean pulmonary arterial pressure and pulmonary vascular resistance.[1] In the World Health Organization (WHO) classification of pulmonary hypertension, precapillary pulmonary hypertension due to lung disease is classified as group 3. The most common lung diseases associated with group 3 pulmonary hypertension are chronic obstructive pulmonary disease and interstitial lung disease.

Pulmonary hypertension has been reported in up to 86% of patients with interstitial lung disease and is associated with reduced exercise capacity, greater need for supplemental oxygen, decreased quality of life, and earlier death.[2-4] Despite the global prevalence and poor clinical course of pulmonary hypertension due to interstitial lung disease, there are currently no approved therapies for these patients. Although data are limited, therapies approved for group 1 pulmonary hyper-tension (pulmonary arterial hypertension) have been used to treat group 3 pulmonary hypertension.[5] Previous studies of vasodilator therapies have shown conflicting results. The largest trial to date evaluated the soluble guanylate cyclase stimulator riociguat in a patient population with group 3 pulmonary hypertension and was stopped early owing to serious harm.[6] Treprostinil is a stable analogue of prostacyclin, which promotes direct vasodilation of pulmonary and systemic arterial vascular beds and inhibits platelet aggregation.[7] An inhaled formulation of treprostinil was previously shown to improve exercise capacity after 12 weeks of therapy in patients with group 1 pulmonary hypertension.[8] Data from previously completed pilot studies suggest that inhaled treprostinil can improve hemodynamics and functional capacity in patients with group 3 pulmonary hypertension.[9-12] Therefore, the objective of the INCREASE trial was to evaluate the safety and efficacy of inhaled treprostinil in patients with pulmonary hypertension due to interstitial lung disease.

Trial Design and Oversight

INCREASE was a multicenter, randomized, double-blind, placebo-controlled trial. The trial was monitored by an independent data and safety monitoring committee and was conducted in accordance with Good Clinical Practice guidelines.

Trial Population

The trial population consisted of patients 18 years of age or older in whom interstitial lung disease was diagnosed on the basis of evidence of diffuse parenchymal lung disease on computed tomography of the chest (not centrally adjudicated) performed within 6 months before randomization. Confirmation of group 3 pulmonary hypertension by right heart catheterization within 1 year before randomization was required. Group 3 pulmonary hypertension was defined by pulmonary vascular resistance of more than 3 Wood units, pulmonary capillary wedge pressure of 15 mm Hg or lower, and mean pulmonary arterial pressure of 25 mm Hg or higher. Patients with group 3 pulmonary hypertension due to connective tissue disease were also required to have a baseline forced vital capacity of less than 70%. Eligible patients also had to walk at least 100 m during a 6-minute walk test. Patients receiving drug treatment (i.e., pirfenidone or nintedanib) for their underlying lung disease were required to have been receiving a stable dose for at least 30 days before undergoing randomization. Patients receiving approved therapy for pulmonary arterial hypertension within 60 days before randomization were not eligible for enrollment. Written informed consent was obtained from all the patients.

TABLE 4

Characteristics of the Patients at Baseline.*

Characteristic	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	All Patients (N = 326)
Female sex - no. (%)	85 (52.1)	68 (41.7)	153 (46.9)
Mean age at randomization (range) - yr	65.6 (26-90)	67.4 (36-85)	66.5 (26-90)
Age distribution - no. (%)			
<65 yr	64 (39.3)	48 (29.4)	112 (34.4)
65 to <80 yr	83 (50.9)	100 (61.3)	183 (56.1)
≥80 yr	16 (9.8)	15 (9.2)	31 (9.5)
Race or ethnic group - no. (%)†			
White	112 (68.7)	126 (77.3)	238 (73.0)
Black or African American	41 (25.2)	30 (18.4)	71 (21.8)
American Indian or Alaska Native	2 (1.2)	1 (0.6)	3 (0.9)
Asian	7 (4.3	5 (3.1)	12 (3.7)
Multiple	0	1 (0.6)	1 (0.3)
Unknown	1 (0.6)	0	1 (0.03)
Hispanic or Latino ethnic group - no. (%)†			
Yes	11 (6.7)	16 (9.8)	27 (8.3)
No	152 (93.3)	146 (89.6)	298 (91.4)
Data missing	0	1 (0.6)	1 (0.3)
Mean time since diagnosis - yr	0.54 ± 1.16	0.54 ± 1.31	0.54 ± 1.23

TABLE 4-continued

Characteristics of the Patients at Baseline.*			
Characteristic	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	All Patients (N = 326)
Cause of lung disease - no. (%)			
Idiopathic interstitial pneumonia	65 (39.9)	81 (49.7)	146 (44.8)
Chronic hypersensitivity pneumonitis	10 (6.1)	9 (5.5)	19 (5.8)
Occupational lung disease	5 (3.1)	1 (0.6)	6 (1.8)
Combined pulmonary fibrosis and emphysema	42 (25.8)	40 (24.5)	82 (25.2)
Connective tissue disease	40 (24.5)	32 (19.6)	72 (22.1)
Other	1 (0.6)	0	1 (0.3)
Idiopathic interstitial pneumonia subcategory - no. (%)			
Idiopathic pulmonary fibrosis	37 (22.7)	55 (33.7)	92 (28.2)
Idiopathic nonspecific interstitial pneumonia	21 (12.9)	16 (9.8)	37 (11.3)
Respiratory bronchiolitis associated with interstitial lung disease	2 (1.2)	0	2 (0.6)
Desquamative interstitial pneumonia	0	1 (0.6)	1 (0.3)
Acute interstitial pneumonia	0	1 (0.6)	1 (0.3)
Unclassified idiopathic interstitial pneumonia	5 (3.1)	8 (4.9)	13 (4.0)
Use of supplemental oxygen - no. (%)	119 (73.0)	114 (69.9)	233 (71.5)
Background therapy - no (%)			
None	133 (81.6)	119 (73.0)	252 (77.3)
Pirfenidone only	19 (11.7)	25 (15.3)	44 (13.5)
Nintedanib only	11 (6.7)	19 (11.7)	30 (9.2)

*Plus-minus values are means ± SD. Additional patient characteristics at baseline are provided in Table S2 in the Supplementary Appendix. Percentages may not total 100 because of rounding.
†Race and ethnic group were reported by the patient.

Trial Procedures

Within 30 days after screening, eligible patients were randomly assigned in a 1:1 ratio to receive inhaled treprostinil (Tyvaso, United Therapeutics) or placebo in a double-blind manner. Randomization, based on permuted blocks, was stratified by baseline 6-minute walk distance (≤350 m vs. >350 m) and was implemented through an interactive Web-response system.

Inhaled treprostinil (0.6 mg per milliliter) was administered by means of an ultrasonic, pulsed-delivery nebulizer at 6 μg per breath. Placebo was administered similarly as a visually identical solution. The first dose of trial drug (3 breaths) was administered in the clinic, followed by at least a 1-hour observation period. The dose of treprostinil or placebo was adjusted, with dose escalation (an additional 1 breath four times daily) occurring as often as every 3 days, with a target dose of 9 breaths four times daily and a maxi-mum dose of 12 breaths four times daily. Investigators adjusted the dose on an individual patient basis to achieve the maximum tolerated dose leading to functional improvement.

Trial Assessments

The 6-minute walk test was performed and laboratory data were obtained at baseline and at weeks 4, 8, 12, and 16, or at the time of early discontinuation of treprostinil or placebo. Each 6-minute walk test was performed 10 to 60 minutes after the most recent dose of active drug or placebo, which is the time of peak plasma treprostinil exposure. A trough test was performed at week 15 at least 4 hours after the participant received a dose of treprostinil or placebo and at least 24 hours before the week 16 test. Pulse oximetry was performed immediately before, during, and after each 6-minute walk test. Measurement of N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels and pulmonary function tests were performed at baseline and at weeks 8 and 16 (or at early discontinuation) after the patients recovered from the 6-minute walk test. The St. George's Respiratory Questionnaire (SGRQ), a quality-of-life measure, was completed at baseline and week 16 or at the time of early discontinuation.

Outcome Measures

The primary end point of the trial was the difference between the two groups in the change in peak 6-minute walk distance from baseline to week 16. Secondary efficacy end points were analyzed in the following hierarchical testing order: the change in NT-proBNP level from baseline to week 16, the time to clinical worsening, the change in 6-minute walk distance at peak plasma treprostinil level at week 12, and the change in 6-minute walk distance at trough treprostinil level at week 15. The time to clinical worsening was evaluated from the time of randomization until the patient's withdrawal from the trial and was defined as the time until the occurrence of any one of the following events: hospitalization for a cardiopulmonary indication, a decrease in 6-minute walk distance greater than 15% from baseline that was directly related to the disease under study at two consecutive visits and at least 24 hours apart, death from any cause, or lung transplantation.

Exploratory end points were the changes in peak 6-minute walk distance at weeks 4 and 8, quality of life as measured with the use of the SGRQ at week 16, and the distance-saturation product (calculated by multiplying the total distance walked by the lowest oxygen saturation measurement during the 6-minute walk) at week 16. Safety end points included adverse events, abnormal laboratory results, oxy-

genation as measured by pulse oximetry (Spo2) and supplemental oxygen requirement, changes in pulmonary function test results, hospitalization for a cardiopulmonary indication, and investigator-reported exacerbations of underlying lung disease, defined as acute, clinically significant respiratory deterioration characterized by evidence of new widespread alveolar abnormality.

Statistical Analysis

Original estimates suggested that with 266 patients randomly assigned in a 1:1 ratio to receive inhaled treprostinil or placebo, the trial would have at least 90% power at a significance level of 0.05 (two-sided) to detect a between-group difference of 30 m in the change in peak 6-minute walk distance from baseline at week 16, assuming a standard deviation of 75 m. To account for approximately 15% of participants discontinuing the trial, 314 patients would need to be enrolled.

For the primary efficacy analysis, the change in 6-minute walk distance was analyzed by mixed-model repeated-measures methods, under the assumption that missing data were missing at random. The model included the change from baseline to peak 6-minute walk distance as the dependent variable, with treatment, week, and treatment-by-week interaction as fixed effects, and the baseline 6-minute walk distance as a covariate. A sensitivity analysis for the primary end point was performed by means of a multiple imputation approach with a multivariate normal imputation model according to the Markov chain Monte Carlo method. The imputation model included treatment group, all scheduled visits, the patient's sex, and the patient's age at randomization. If the result for the primary efficacy end point was significant, secondary efficacy end points were to be evaluated according to a hierarchical testing procedure. Confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects for secondary efficacy end points.

Results

Patients

Of 462 patients screened for eligibility, 326 were enrolled at 93 centers and were randomly assigned to receive placebo (163 patients) or inhaled treprostinil (163 patients) (FIG. **2**). Baseline characteristics were similar in the two groups (Table 4). The mean age of the patients was 66.5 years, 46.9% were female, and the most common diagnosis was idiopathic interstitial pneumonia (in 44.8%). At baseline, the mean 6-minute walk distance was 259.6 m, the mean pulmonary vascular resistance was 6.2 Wood units, and the mean NT-proBNP level was 1832.9 pg per milliliter.

Exposure and Follow-up

Patients in the treprostinil group took a median of 11 breaths from the inhaler (66 μg) at each of four daily sessions at week 12 and 12 breaths (72 μg) per session at week 16. The percentage of patients in this group who took 10 to 12 breaths (60 to 72 μg) per session was 57.0% at week 12 and 57.8% at week 16. Patients in the placebo group took a median of 12 breaths from the inhaler per session at weeks 12 and 16.

Forty patients assigned to receive inhaled treprostinil (24.5%) and 38 assigned to placebo (23.3%) discontinued the assigned regimen pre-maturely. These patients were encouraged to remain in the trial and complete assessments through week 16; 33 patients in the treprostinil group and 35 in the placebo group discontinued participation in the trial. The reasons for discontinuation are shown in FIG. **2**.

Primary End Point

Mean within-group changes in the 6-minute walk distance are shown in FIG. **2**. Mixed-model repeated-measures analysis showed that the least-squares mean difference between the treprostinil group and the placebo group in the change from baseline in peak 6-minute walk distance was 31.12 m (95% confidence interval [CI], 16.85 to 45.39; P<0.001) (Table 5 and FIG. **4**). Similar effects were observed across subgroups, including subgroups defined by disease cause and severity (as measured by baseline 6-minute walk distance), baseline hemodynamics, and dose group (FIG. **5**). In addition, the between-group difference in the change from baseline in peak 6-minute walk distance at week 16 was significant when analyzed with multiple imputation according to the Markov chain Monte Carlo method (30.97 m; 95% CI, 16.53 to 45.41; P<0.001) (FIG. **6**).

TABLE 5

Summary of Primary and Secondary End Points.*

End Point	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	Treatment Effect (95% CI)	P Value
Primary end point				
Change in peak 6-minute walk distance from baseline to wk 16 - m†	21.08 ± 5.12	−10.04 ± 5.12	31.12 ± 7.25 (16.85 to 45.39)‡	<0.001
Secondary end points§				
Change in plasma concentration of NT-proBNP from baseline to wk 16¶				
Mean (±SD) change - pg/ml	−396.35 ± 1904.90	1453.95 ± 7296.20		
Median - pg/ml	−22.65	20.65		
Range - pg/ml	−11,433.0 to 5373.1	−5483.3 to 87,148.3		
Ratio to baseline	0.85 ± 0.06	1.46 ± 0.11	±0.58 ± 0.06 (0.47 to 0.72)‖	<0.001

TABLE 5-continued

Summary of Primary and Secondary End Points.*

End Point	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	Treatment Effect (95% CI)	P Value
Occurrence of clinical worsening - no. (%)			0.61 (0.4 to 0.92)**	0.04
Any event	37 (22.7)	54 (33.1)		
Hospitalization for cardiopulmonary indication	18 (11.0)	24 (14.7)		
Decrease in 6 minute walk distance of >15% from baseline	13 (8.0)	26 (16.0)		
Death from any cause	4 (2.5)	4 (2.5)		
Lung transplantation	2 (1.2)	0		
Least-squares mean change in peak 6- minute walk distance from baseline to wk 12 - m†	18.77 ± 4.99	−12.52 ± 5.01	31.29 ± 7.07 (17.37 to 45.21)‡	<0.001
Least-squares mean change in trough 6- minute walk distance from baseline to wk 15 - m	9.3 ± 5.5	−12.7 ± 5.5	21.99 ± 7.7± (6.85 to 37.14)†	0.005††

*Plus-minus values are means ± SE, unless otherwise indicated. For secondary end points, the confidence intervals (CIs) have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects. NT-proBNP denotes N-terminal pro-B-type natriuretic peptide.
†The effect of inhaled treprostinil as compared with placebo on the change in 6-minute walk distance was evaluated with the use of a mixed-model repeat measurement with the change from baseline in peak 6-minute walk distance as the dependent variable; treatment, week, and treatment-by-week interaction as the fixed effects; baseline 6-minute walk distance as the covariate; and subject as the random effect. Results are shown in Figures S1 and S3.
‡This is a least-squares mean difference between the groups.
§The effect of inhaled treprostinil as compared with placebo on the change in log-transformed NT-proBNP was evaluated with the use of a mixed-model repeat measurement with the change from baseline in log-transformed NT-proBNP as the dependent variable; treatment, week, and treatment-by-week interaction as the fixed effects; and log-transformed baseline NT-proBNP as the covariate. Ratio to baseline is the least-squares mean of the change from baseline in log-transformed data.
¶The change in plasma concentration of NT-proBNP from baseline to week 16 was assessed in 156 patients in the treprostinil group and 160 in the placebo group.
‖This is the treatment ratio, which is the ratio of ratios between two treatment groups.
**This is a hazard ratio, calculated from a Cox proportional-hazards model. The P value was calculated with the use of a log-rank test stratified by the baseline 6-minute walk distance category.
††The P value was obtained from 100 multiple imputations with Markov chain Monte Carlo estimation with the use of analysis of covariance (ANCOVA) modeling, with the change from baseline in peak 6-minute walk distance as the dependent variable, treatment as a fixed effect, and baseline 6-minute walk distance as a covariate.

Secondary and Exploratory End Points

Patients assigned to inhaled treprostinil, as compared with those assigned to placebo, showed significant improvements in each of the secondary end points (Table 5). The NT-proBNP level decreased 15% from baseline with inhaled treprostinil and increased 46% from baseline with placebo, as assessed by the least-squares mean for the log-transformed ratio to the baseline level at week 16 (treatment ratio, 0.58; 95% CI, 0.47 to 0.72; P<0.001) (FIG. 7). Clinical worsening occurred in 37 patients (22.7%) in the treprostinil group, as compared with 54 patients (33.1%) in the placebo group (hazard ratio, 0.61; 95% CI, 0.40 to 0.92; P=0.04 by the log-rank test) (FIG. 1). The least-squares mean change from baseline to week 12 in peak 6-minute walk distance was 31.29 m greater in the treprostinil group than in the placebo group (P<0.001), and the change from baseline to week 15 in trough 6-minute walk distance was 21.99 m greater in the treprostinil group (P=0.004). There was no significant between-group difference in patient-reported quality of life as assessed with the SGRQ or in the distance-saturation product at week 16.

Safety End Points

TABLE 6

Summary of Adverse Events

Variable	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	P Value*
Total no. of adverse events	890	793	
Patients with ≥1 adverse event - no. (%)	152 (93.3)	149 (91.4)	0.68
Total no. of serious adverse events†	53	89	
Patients with ≥1 serious adverse event - no. (%)	38 (23.3)	42 (25.8)	0.70
Total no. of adverse events leading to withdrawal of treprostinil or placebo	47	38	
Most frequently occurring adverse events - no. of patients (%)‡			
Cough	71 (43.6)	54 (33.1)	0.07
Headache	45 (27.6)	32 (19.6)	0.12
Dyspnea	41 (25.2)	51 (31.3)	0.27
Dizziness	30 (18.4)	23 (14.1)	0.37
Nausea	25 (15.3)	26 (16.0)	>0.99

TABLE 6-continued

Summary of Adverse Events

Variable	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	P Value*
Fatigue	23 (14.1)	23 (14.1)	>0.99
Diarrhea	22 (13.5)	19 (11.7)	0.74
Throat irritation	20 (12.3)	6 (3.7)	0.007
Oropharyngeal pain	18 (11.0)	4 (2.5)	0.003
NT-proBNP increased	9 (5.5)	25 (15.3)	0.006

*P values were calculated with the use of Fisher's exact test.

‡Shown are the most frequently occurring adverse events occurring in more than 10% of patients in either group in the safety population, which comprised all patients who underwent randomization and received at least one dose of treprostinil or placebo.

The most frequently reported adverse events were cough, headache, dyspnea, dizziness, nausea, fatigue, and diarrhea (Table 6). Most of these events were of mild-to-moderate intensity.

Serious adverse events occurred in 23.3% of the patients who received inhaled treprostinil and in 25.8% of those who received placebo. No serious adverse events were reported significantly more frequently in the treprostinil group than in the placebo group.

Significantly fewer patients in the treprostinil group than in the placebo group had exacerbations of underlying lung disease (43 [26.4%] vs. 63 [38.7%]; P=0.02 by Fisher's exact test). Fewer patients in the treprostinil group than in the placebo group had a first occurrence of clinical worsening that involved hospitalization for a cardiopulmonary indication (18 [11.0%] vs. 24 [14.7%]; P=0.41). Inhaled treprostinil had no deleterious effect on any pulmonary function test variable during the trial. There were no significant treatment-related changes in pulse oximetry or supplemental oxygen use in either group over the trial period.

Discussion

Pulmonary hypertension frequently complicates the treatment of patients with interstitial lung disease and is associated with worse functional status, greater need for supplemental oxygen, and worse outcomes.[3, 13] In the INCREASE trial, patients treated with inhaled treprostinil had significant improvements in exercise capacity, as evidenced by changes in the 6-minute walk distance. Treatment with inhaled treprostinil was also associated with a lower risk of clinical worsening than that in patients who received placebo, as well as reductions in NT-proBNP levels and fewer exacerbations of underlying lung disease, over the 16-week treatment period. The safety profile of inhaled treprostinil observed in this vulnerable patient population was similar to that reported in previous studies. The most frequently reported adverse events were cough, headache, dyspnea, dizziness, nausea, fatigue, and diarrhea. The use of inhaled treprostinil was not associated with any decrement in lung function.

Patients with group 3 pulmonary hypertension are often treated with systemic pulmonary vasodilators, which are currently approved only for treatment of group 1 pulmonary hypertension. However, there is concern that such agents could worsen ventilation-perfusion matching in patients with group 3 pulmonary hypertension. Inhaled agents have the advantage of preferentially redirecting blood flow to the best-ventilated lung units, thus reducing the risk of ventilation-perfusion mismatching.[9, 14] Indeed, a retrospective study of inhaled treprostinil in patients with group 3 pulmonary hypertension showed that such patients had improvements in functional class and 6-minute walk distance without any adverse effect on peripheral oxygen saturation, reinforcing the concept of unchanged or even improved ventilation-perfusion matching with inhaled treprostinil.[10] Similarly, in the current trial, we found no evidence of worsened oxygenation, which further allays concerns about ventilation-perfusion mismatching.

The INCREASE trial was not without its limitations. The trial was of short duration, and 21% of the patients discontinued the trial prematurely (before week 16). In addition, events of clinical worsening and exacerbation of underlying lung disease were investigator-reported and not adjudicated by an independent review committee. Finally, the size of the favorable treatment effect on the 6-minute walk distance with inhaled treprostinil is similar to estimates of the minimum clinically important difference for this test in patients with pulmonary disease (21.7 to 37 m in a study by Nathan et al., and 24 to 45 m in a study by du Bois et al.).[15, 16]

This study showed that among patients with pulmonary hypertension due to interstitial lung disease, treatment with inhaled treprostinil improved exercise capacity as shown by improvement in the 6-minute walk distance through the end of the 16-week treatment period. In addition, treatment with inhaled treprostinil was associated with a lower risk of clinical worsening than that with placebo, a reduction in NT-proBNP levels, and fewer exacerbations of underlying lung disease.

Supplemental Information

TABLE 7

Additional Baseline Patient Characteristics.

	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	All Patients (N = 326)
6-minute walk distance, meters; mean (range) Median	254.1 (100-538) 256.0	265.1 (30-505) 260.0	259.6 (30-538) 259.0
Pulmonary vascular resistance, Woods units; mean (range) Median	6.369 (3.11-8.05) 5.570	6.013 (3.06-17.62) 5.060	6.191 (3.06-18.05) 5.275
NT-proBNP, pg/mL; mean (range)	1857.53 (10.2-21942.0)	1808.86 (23.0-16297.0)	1832.88 (10.2-21942.0)

TABLE 7-continued

Additional Baseline Patient Characteristics.

	Inhaled Treprostinil (N = 163)	Placebo (N = 163)	All Patients (N = 326)
Median*	550.50	420.80	503.85
Pulmonary arterial pressure, mmHg; mean (range	37.2 (25-74)	36.0 (25-61)	36.6 (25-74)
Median	35.0	35.0	35.0
Pulmonary capillary wedge pressure, mmHg; mean (range)	10.1 (2-20)	9.6 (0-15)	9.8 (0-20)
Median	10.0	10.0	10.0
Pulmonary function tests			
FEV1% Predicted; mean (range)	63.9 (23, 120)	65.0 (22, 145)	
Median	63.0	63.0	
FVC % Predicted; mean (range)	62.5 (24, 130)	63.8 (20, 134)	
Median	60.0	61.0	
TLC % Predicted; mean (range)	62.9 (25, 126)	64.2 (30, 109)	
Median	62.0	62.5	
DLCO % Predicted; mean (range)	30.0 (5, 86)	28.1 (1. 86)	
Median	29.0	26.0	

DLCO, lung diffusion capacity;
FEV1, forced expiratory volume in 1 second;
FVC, forced vital capacity;
NT-proBNP, N-terminal pro-brain natriuretic peptide;
TLC, total lung capacity
*N = 156 inhaled treprostinil; N = 160 placebo

TABLE 8

St. George's Respiratory Questionnaire Results.

	Inhaled Treprostinil N = 163		Placebo N = 163	
Visit Statistic	Value	Change from Baseline	Value	Change from Baseline
Baseline				
n	143		134	
Mean (SD)	57.17 (15.77)		57.67 (15.78)	
Median	59.80		56.30	
Interquartile	45.60, 67.90		46.50 70.70	
Min, Max	14.7, 94.9		18.4 88.6	
Week 16				
n	143	143	134	134
Mean (SD)	55.91 (17.07)	−1.25 (10.99)	57.49 (15.33)	−0.18 (10.72)
Median	56.30	−0.70	55.50	0.10
Interquartile	40.50, 67.00	−7.10, 5.20	46.80 69.70	−6.50, 6.10
Min, Max	3.5, 92.0	−40.4, 29.0	16.9 96.5	−31.9, 33.3
LS Mean (SE)		−1.30 (0.87)		−0.13 (0.90)
LS Mean Difference (SE) and (95% CI)		−1.18, (1.25) (−3.63, 1.28)		

ANCOVA, analysis of covariance;
CI, confidence interval;
LS Mean, least squares mean;
SD, standard deviation;
SE, standard error

The St. George's Respiratory Questionnaire has a range of results from 0 to 100, with higher scores indicating greater impairment and with a minimum clinically important difference of 4 points.

The changes from baseline in Total Score and each of the 3 domain scores were analyzed by parametric ANCOVA with no imputation for missing data.

The confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects.

TABLE S4

Distance Saturation Product Results by Study Visit (m %).

Visit/Variable Statistic	Inhaled Treprostinil N = 163	Placebo N = 163
Baseline		
n	118	109
Mean (SD)	208.140 (81.130)	218.247 (77.405)
Median	201.320	215.760
Interquartile	150.060, 256.750	170.800, 268.800
Min, Max	77.04, 421.07	63.00, 417.35
Week 16 Change from Baseline		
n	118	109
Mean (SD)	7.607 (45.680)	−4.803 (53.026)
Median	8.385	−1.950
Interquartile	−12.960, 34.890	−38.180, 32.000
Min, Max	−217.26, 117.42	−184.85, 129.28
LS Mean (SE)	7.2 (4.5)	−4.3 (4.7)
LS Mean Difference (SE) and 95% CI	11.51 (6.5), 95% CI (−1.33, 24.35)	

ANCOVA, analysis of covariance;
CI, confidence interval;
LS Mean, least squares mean;
SD, standard deviation;
SE, standard error;
SpO_2, saturation of peripheral capillary oxygenation

Change in distance saturation product is the product of distance walked and lowest SpO_2 recorded during the 6-minute walk test.[7] Change from baseline to Week 16 in distance saturation product was analyzed by parametric ANCOVA with no imputation for missing distance saturation product values.

The confidence intervals have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects.

TABLE 9

Serious Adverse Events by Preferred Term

Serious Adverse Events	Inhaled treprostinil N = 163 n	Placebo N = 163 n
Any Serious Event	53 events in 38 patients (23.3%)	89 events in 42 patients (25.8%)
Acute respiratory failure	4	5
Death with unknown cause	3	1
Dyspnoea	3	7
Interstitial lung disease	3	2
Bronchitis	2	1
Chronic obstructive pulmonary disease	2	2
Chronic respiratory failure	2	0
Respiratory failure	2	5
Upper respiratory tract infection	2	1
Acute myocardial infarction	1	2
Acute right ventricular failure	1	0

TABLE 9-continued

Serious Adverse Events by Preferred Term

Serious Adverse Events	Inhaled treprostinil N = 163 n	Placebo N = 163 n
Arrhythmia	1	0
B-cell lymphoma	1	0
Bronchopulmonary aspergillosis	1	0
Cardiac arrest	1	2
Cardiac failure congestive	1	2
Cardiopulmonary failure	1	0
Cellulitis	1	0
Cerebral haemorrhage	1	0
Chest pain	1	1
Combined pulmonary fibrosis and emphysema	1	0
Cor pulmonale	1	0
Haemoptysis	1	0
Hyperglycaemia	1	0
Hypervolaemia	1	0
Hypoxia	1	0
Idiopathic pulmonary fibrosis	1	4
Influenza	1	1
Left ventricular failure	1	0
Pain in extremity	1	0
Pneumonia	1	9
Pneumothorax	1	1
Pulmonary hypertension	1	1
Pulmonary oedema	1	0
Rhinovirus infection	1	0
Right ventricular failure	1	2
Syncope	1	1
Tachycardia	1	0
Abdominal pain	0	2
Acute kidney injury	0	1
Aspiration	0	1
Atrial fibrillation	0	1
Bradycardia	0	1
Cardiac failure	0	2
Cardiac failure acute	0	1
Cardiogenic shock	0	1
Chronic right ventricular failure	0	1
Coagulopathy	0	1
Cor pulmonale acute	0	1
Coronary artery disease	0	1
Disease progression	0	2
Epistaxis	0	1
Fluid overload	0	4
Haematochezia	0	1
Hypertension	0	1
Lumbar vertebral fracture	0	1
Metabolic encephalopathy	0	1
Pain	0	1
Pneumonia influenzal	0	1
Post procedural infection	0	1
Presyncope	0	2
Pulmonary congestion	0	1
Respiratory distress	0	1
Scleroderma	0	1
Sepsis	0	2
Transplant dysfunction	0	1
Urosepsis	0	1

TABLE 10

Analysis of Lung Function Test Parameters Using Mixed Model Repeated Measurement.

Variable Visit Treatment	N	LS Mean	Contrast: Inhaled treprostinil – Placebo Estimated Difference (95% CI)	P-value
FVC (mL)				
Week 8				
Inhaled treprostinil	142	5.49	28.47	0.35
Placebo	141	−22.98	(−30.81, 87.74)	
Week 16				
Inhaled treprostinil	130	9.77	44.40	0.21
Placebo	126	−34.63	(−25.25, 114.05)	
FVC (% predicted)				
Week 8				
Inhaled treprostinil	142	0.77	1.79	
Placebo	141	−1.02	(0.37, 3.21)	0.01
Week 16				
Inhaled treprostinil	130	1.07	1.80	0.03
Placebo	126	−0.72	(0.20, 3.39)	
FEV1 (mL)				
Week 8				
Inhaled treprostinil	142	−21.34	−8.95	0.72
Placebo	141	−12.39	(−57.16, 39.26)	
Week 16				
Inhaled treprostinil	130	−32.18	−2.56	0.93
Placebo	126	−29.62	(−57.67, 52.55)	
FEV1 (% predicted)				
Week 8				
Inhaled treprostinil	142	−0.18	0.57	0.43
Placebo	141	−0.75	(−0.83, 1.96)	
Week 16				
Inhaled treprostinil	130	−0.24	0.38	0.65
Placebo	126	−0.62	(−1.25, 2.01)	
TLC (mL)				
Week 8				
Inhaled treprostinil	135	−38.75	−16.23	0.80
Placebo	136	−22.51	(−141.9, 109.41)	
Week 16				
Inhaled treprostinil	127	45.43	17.37	0.85
Placebo	116	28.06	(−158.9, 193.61)	
TLC (% predicted)				

TABLE 10-continued

Analysis of Lung Function Test Parameters Using Mixed Model Repeated Measurement.

Variable Visit Treatment	N	LS Mean	Contrast: Inhaled treprostinil – Placebo Estimated Difference (95% CI)	P-value
Week 8				
Inhaled treprostinil	135	−0.05	0.28	0.76
Placebo	136	−0.32	(−1.49, 2.05)	
Week 16				
Inhaled treprostinil	127	2.52	1.49	0.34
Placebo	116	1.03	(−1.57, 4.54)	
DLCO (mL/min/mmHg)				
Week 8				
Inhaled treprostini	136	−0.27	0.19	0.56
Placebo	136	−0.47	(−0.45, 0.84)	
Week 16				
Inhaled treprostinil	128	−0.61	0.02	0.96
Placebo	112	−0.63	(−0.73, 0.76)	
DLCO (% predicted)				
Week 8				
Inhaled treprostinil	136	−0.13	1.07	0.13
Placebo	136	−1.20	(−0.32, 2.47)	
Week 16				
Inhaled treprostinil	128	−1.14	0.60	0.44
Placebo	112	−1.74	(−0.93, 2.14)	

CI, confidence interval;
DLCO, diffusing capacity of the lungs for carbon monoxide;
FEV1, forced expiratory volume in 1 second;
FVC, forced vital capacity;
TLC, total lung capacity;,
LS Mean, least squares mean;
SE, standard error;
TLC, total lung capacity

LS Mean (SE), P-values, estimated difference (SE), and associated 95% CIs are from the mixed model repeated measurement with the change from Baseline in pulmonary function test parameter as the dependent variable; treatment, week, treatment by week interaction as the fixed effects; Baseline measurement as the covariate; and subject as the random effect. An unstructured variance/covariance structure shared across treatment groups was used to model the within-subject errors.

The confidence intervals and p-values have not been adjusted for multiplicity and cannot be used to infer definitive treatment effects.

TABLE 11

SpO_2 (%) Measured by Pulse Oximetry Results at Baseline and Week 16.

	Inhaled Treprostinil N = 163		Placebo N = 163		
Visit Statistic	Value	Change from Pre- walk	Value	Change from Pre- Walk	P-value*
Baseline Pre-walk SpO_2 (%)					
n	163		162		
Mean (SD)	95.3 (3.95)		94.5 (4.81)		
Median	96.0		96.0		
Min, Max	72, 100		68, 100		

TABLE 11-continued

SpO_2 (%) Measured by Pulse Oximetry Results at Baseline and Week 16.

Visit Statistic	Inhaled Treprostinil N = 163 Value	Inhaled Treprostinil N = 163 Change from Pre- walk	Placebo N = 163 Value	Placebo N = 163 Change from Pre- Walk	P-value*
Baseline During Walk SpO_2 (%)					
n	154	154	153	153	0.13
Mean (SD)	80.3 (8.22)	−15.0 (7.87)	78.5 (8.20)	−16.1 (7.76)	
Median	81.0	−14.0	78.0	−15.0	
Min, Max	53, 99	−41, 2	53, 98	−39, 4	
Baseline Post-walk SpO_2 (%)					
n	163	163	162	162	0.17
Mean (SD)	85.3 (7.31)	−9.9 (6.50)	83.7 (8.74)	−10.9 (8.06)	
Median	86.0	−10.0	83.5	−11.0	
Min, Max	59, 100	−26, 5	57, 99	−39, 7	
Week 16 Pre-walk SpO_2 (%)					
n	130		122		
Mean (SD)	94.5 (4.35)		94.5 (4.22)		
Median	95.0		95.0		
Min, Max	74, 100		78, 100		
Week 16 During Walk SpO_2 (%)					
n	123	123	114	114	0.27
Mean (SD)	76.8 (7.70)	−17.6 (7.01)	78.2 (9.28)	−16.6 (9.04)	
Median	77.0	−17.0	79.0	−16.0	
Min, Max	46, 99	−38, −1	28, 98	−61, −1	
Week 16 Post-walk SpO_2 (%)					
n	128	128	122	122	0.07
Mean (SD)	82.1 (9.24)	−12.4 (8.05)	83.7 (7.75)	−10.8 (7.09)	
Median	83.0	−13.0	84.0	−11.5	
Min, Max	51, 100	−29, 3	65, 100	−31, 6	

SD, standard deviation;

SpO_2, saturation of peripheral capillary oxygenation

*P-values are calculated from analysis of covariance with change from pre-walk as dependent variable, treatment as fixed effect, and baseline SpO_2 as covariate.

TABLE 12

Supplemental Oxygen Use (L/min) at Baseline and Week 16.

Visit Statistic	Inhaled Treprostinil N = 163 Value	Inhaled Treprostinil N = 163 Change from Baseline	Placebo N = 163 Value	Placebo N = 163 Change from Baseline	P-value*
Baseline Pre-walk (L/min)					
n	163		163		
Mean (SD)	2.7 (2.2)		2.4 (2.0)		
Median	3.0		2.0		
Min, Max	0, 10		0, 8		
Baseline During Walk (L/min)					
n	163		163		
Mean (SD)	4.9 (4.0)		4.5 (3.8)		
Median	4.0		4.0		
Min, Max	0, 25		0, 15		
Week 16 Pre-walk (L/min)					
n	131	131	129	129	0.18
Mean (SD)	3.0 (2.5)	0.4 (1.4)	2.9 (2.4)	0.6 (1.3)	
Median	3.0	0.0	3.0	0.0	
Min, Max	0, 10	−3, 6	0, 10	−3, 5	

TABLE 12-continued

Supplemental Oxygen Use (L/min) at Baseline and Week 16.

Visit Statistic	Inhaled Treprostinil N = 163 Value	Inhaled Treprostinil N = 163 Change from Baseline	Placebo N = 163 Value	Placebo N = 163 Change from Baseline	P-value*
Baseline During Walk (L/min)					
n	129	129	123	123	0.39
Mean (SD)	4.9 (4.0)	0.1 (0.8)	4.6 (3.7)	0.1 (0.3)	
Median	4.0	0.0	4.0	0.0	
Min, Max	0, 25	−2, 8	0, 15	0, 3	

SD, standard deviation

Subjects who did not use supplemental oxygen were coded as 0 in the summaries.

Subjects who received supplemental oxygen during the Baseline 6-minute walk test continued to receive the same flow rate at all subsequent 6-minute walk test assessments.

*P-values are calculated from analysis of covariance with change from baseline as dependent variable, treatment as fixed effect, and baseline oxygen use as covariate.

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Example 4. Aerosolized and Powder Inhaled Treprostinil

Randomized, 6-treatment, 6-period, 6-sequence, cross-over study (6×6 Williams design) in 36 healthy volunteers was performed to compare nebulized inhaled treprostinil administered by Tyvaso® nebulizer and Treprostinil inhalation powder (TreT) administered via a dry powder inhaler (published US Patent Application 20190321290). 4 subjects discontinued the study early (COVID-19, n=2; withdrawal by subject, n=1; non-compliance with study requirements, n=1).

Tyvaso Dose	TreT Dose
18 μg (3 nebulizer breaths)	16 μg cartridge
54 μg (9 nebulizer breaths)	48 μg cartridge
72 μg (12 nebulizer breaths)	64 μg cartridge

TABLE 14

Pharmacokinetic results for various doses for Tyvaso and TreT administered treprostinil. See also FIG. 9 and 10.

Comparison	Parameter	Geometric LSM (TreT) [CV %]	Geometric LSM (Tyvaso) [CV %]	Geometric LSM Ratio (%) [TreT/Tyvaso]	90% Confidence Interval
TreT 16 μg vs. Tyvaso 18 μg	AUC0-5	0.268 [24.1%]	0.233 [44.1%]	115	(104.59, 127.42)
	Cmax	0.377 [26.6%]	0.291 [59.8%]	130	(115.55, 145.95)
TreT 48 μg vs. Tyvaso 54 μg	AUC0-5	0.766 [21.8%]	0.757 [42.5%]	101	(91.63, 111.65)
	Cmax	1.07 [28.9%]	0.764 [53.4%]	139	(124.13, 156.73)
TreT 64 μg vs. Tyvaso 72 μg	AUC0-5	0.937 [23.8%]	1.02 [41.9%]	91.5	(83.16, 100.78)
	Cmax	1.27 [28.5%]	1.02 [54.7%]	124	(110.56, 139.61)

TABLE 15

Adverse events for various doses for Tyvaso and TreT administered treprostinil.

	TreT 16 μg N = 34 n (%)	Tyvaso 18 μg N = 34 n (%)	TreT 48 μg N = 34 n (%)	Tyvaso 54 μg N = 34 n (%)	TreT 64 μg N = 33 n (%)	Tyvaso 72 μg N = 35 n (%)
Adverse Events	16 (47.1)	13 (38.2)	23 (67.6)	21 (61.8)	22 (66.7)	25 (71.4)
Cough	15 (44.1)	11 (32.4)	20 (58.8)	18 (52.9)	21 (63.6)	24 (68.6)
Headache	2 (5.9)	3 (8.8)	4 (11.8)	7 (20.6)	6 (18.2)	6 (17.1)
Throat irritation	1 (2.9)	1 (2.9)	3 (8.8)	5 (14.7)	3 (9.1)	4 (11.4)
Dizziness	1 (2.9)	2 (5.9)	1 (2.9)	4 (11.8)	2 (6.1)	2 (5.7)
Nausea	0	0	0	2 (5.9)	2 (6.1)	1 (2.9)
Chest discomfort	1 (2.9)	0	3 (8.8)	2 (5.9)	0	2 (5.7)

Conclusions

AUC0-5 was generally comparable for each TreT and Tyvaso dose level. Cmax values for TreT were slightly higher than Tyvaso Cmax values across dose comparisons. AE profile consistent with known prostacyclin effects and previous studies of Tyvaso. Between-subject variability for both AUC0-5 and Cmax was approximately two-fold less for TreT compared to Tyvaso. AUC0-5 and Cmax for TreT and Tyvaso increased in an approximately dose-proportional manner. Median Tmax: ~10 minutes for TreT and ~10 to 15 minutes with Tyvaso.

Example 5. Aerosolized and Powder Inhaled Treprostinil. Safety Evaluation

Primary Objective

To evaluate the safety and tolerability of Treprostinil Inhalation Powder (TreT) administered by a dry powder inhaler, such as the one shown in FIG. **11**, in subjects with pulmonary arterial hypertension (PAH) currently treated with Tyvaso® (treprostinil inhalation solution administered via a nebulizer)

Secondary Objectives

To evaluate systemic exposure and pharmacokinetics (PK) of treprostinil in subjects with PAH when delivered as Tyvaso® and TreT. To evaluate 6-Minute Walk Distance (6MWD) at study entry and after 3 weeks of treatment with TreT. To evaluate subject satisfaction with and preference for TreT with the Preference Questionnaire for Inhaled Treprostinil Devices (PQ-ITD). To evaluate patient reported PAH symptoms and impact with the PAH-Symptoms and Impact Questionnaire (PAH-SYMPACT).

Eligibility Criteria

Diagnosis of WHO Group I PAH.

Subject must have started Tyvaso≥3 months prior to Baseline and on a stable regimen (no change in dose within 30 days of Baseline Visit) of Tyvaso (6 to 12 breaths QID).

Background therapy for PAH (eg, endothelin receptor antagonist or phosphodiesterase-5-inhibitor or both), on stable dose for a minimum of 30 days prior to Screening. Exclude other prostacyclin analogue or agonist (selexipag, epoprostenol, iloprost, or beraprost).

Excluding subjects with WHO Functional Class IV at Screening.

Subject is not able to perform inhalation maneuvers that meet inspiratory training criteria.

Exclude conditions which limits ambulation or ability to complete 6MWT (Baseline 6MWD>150 m).

Excluded initiation of pulmonary rehabilitation within 12 weeks prior to the Baseline Visit.

FIG. **12** shows a design of the study. Table 16 presents information relating Tret and Tyvaso doses.

TABLE 16

Tyvaso dose (QID)	TreT Dose (QID)	Device usage
6 to 7 breaths	32 μg	32 μg cartridge
8 to 10 breaths	48 μg	48 μg cartridge
11 to 12 breaths	64 μg	32 μg + 32 μg cartridges

TABLE 17

Baseline demographics	
Age (years)	
Median	57.0 (range: 23-82)
Sex, n (%)	
Female	43 (84.3)
Male	8 (15.7)
Current PAH Diagnosis, n (%)	
Idiopathic/familial	29 (56.9)
Associated with unrepaired/repaired congenital shunts	4 (7.8)
Associated with collagen vascular disease	14 (27.5)
Associated with HIV	1 (2.0)
Associated with appetite suppressant/ other drug or toxin use	3 (5.9)
WHO Functional Class at Screening, n (%)	
I	6 (11.8)
II	31 (60.8)
III	14 (27.5)

TABLE 12

Summary of Subject Accountability

	TreT Dose in Treatment Phase			
	32 mcg N = 2 n (%)	48 mcg N = 27 n (%)	64 mcg N = 22 n (%)	Overall N = 51 n (%)
Number of Subjects Enrolled	2	27	22	51
Received TreT	2 (100.0)	27 (100.0)	22 (100.0)	51 (100.0)
Enrolled in Optional Extension Phase	2 (100.0)	26 (96.3)	21 (95.5)	49 (96.1)
Subjects Who Discontinued Treatment Phase	0	1 (3.7)	1 (4.5)	2 (3.9)
Adverse Event	0	1 (3.7)	1 (4.5)	2 (3.9)
Subjects Who Discontinued OEP*	0	3 (11.1)	0	3 (5.9)
Adverse Event	0	2 (7.4)	0	2 (3.9)
Lost to Follow-up	0	1 (3.7)	0	1 (2.0)

TABLE 13

Summary of background PAH medication

	Overall N = 51; n (%)
ERA	43 (84.3%)
Ambrisentan	24 (47.1%)
Bosentan	2 (3.9%)
Macitentan	17 (33.3%)
PDE5-I	41 (80.4%)
Sildenafil	17 (33.3%)
Tadalafil	24 (47.1%)
sGC	7 (13.7%)
Riociguat	7 (13.7%)

Of the 51 subjects enrolled, assigned TreT doses for 3-week treatment period were 32 μg for 2 subjects; 48 μg for 27 subjects; 64 μg for 22 subjects. 49 subjects rolled into the Optional Extension Phase (OEP). FIG. **13** shows a number of subjects for various maintenance TreT doses in the OEP.

FIG. **14** shows a change in 6 minute walk distance (6MWD) with respect to a baseline 6MWD as a function of duration of TreT treatment. The change from Baseline in 6MWD for TreT overall demonstrated a significant improvement (11.5 m increase; p=0.0217) at Week 3. The improvements in 6MWD for TreT overall were sustained in the Optional Extension Phase.

Patient Reported Outcome Measures

The PQ-ITD is a patient-reported outcome questionnaire to evaluate subject satisfaction with and preference for inhaled treprostinil devices. The PQ-ITD was given at Baseline to evaluate the Tyvaso Inhalation System and at Week 3 to evaluate the TreT Inhaler.

The distribution of responses to each question on the PQ-ITD was significantly improved (p≤0.0003) between Baseline (Tyvaso nebulizer) and Week 3 (TreT inhaler). Overall satisfaction with the TreT inhaler was significantly improved at Week 3 (95.7%, p<0.0001) compared to satisfaction with the Tyvaso nebulizer at Baseline, FIG. **14**.

PAH SYMPACT

The PAH-SYMPACT is a well validated patient-reported outcome questionnaire given to assess PAH symptoms and effects. The PAH-SYMPACT contains four domains (Cardiopulmonary Symptoms, Cardiovascular Symptoms, Physical Impacts, Cognitive/Emotional Impacts) and was given at Baseline, Week 3, and Week 11.

Analysis of patient-reported PAH SYMPACT data revealed a trend of improvement at both Week 3 and Week 11 for subjects receiving TreT.

Mean change from Baseline was lower for all domain scores of the PAH-SYMPACT at both weeks (range: −0.05 to −0.22), with significant improvements for physical impacts (range: −1.1 to 1.0; p=0.0438) and cognitive/emotional impacts (range: −1.3 to 0.5; p=0.0048) at Week 3.

TABLE 18

Overall Safety

	TreT Dose in Treatment Phase			
Treatment Phase	32 mcg N = 2 n (%)	48 mcg N = 27 n (%)	64 mcg N = 22 n (%)	Overall N = 51 n (%)
Total number of AEs	0	37	22	59
Total number of SAEs	0	1	1	2
AEs leading to withdrawal of study drug	0	1	1	2
Optional Extension Phase	0			
Total number of AEs	2	51	29	82
Total number of SAEs	0	10	4	14
AEs leading to withdrawal of study drug	0	3	0	3

TABLE 19

Most frequent adverse events during the treatment phase

	Treatment Phase Dose				TRIUMPH	
Preferred Term	32 mcg N = 2 n (%)	48 mcg N = 27 n (%)	64 mcg N = 22 n (%)	Overall N = 51 n (%)	Tyvaso n (%)	Placebo n (%)
Cough	0	9 (33.3)	4 (18.2)	13 (25.5)	62 (54)	35 (29)
Headache	0	4 (14.8)	4 (18.2)	8 (15.7)	47 (41)	27 (23)

TABLE 19-continued

Most frequent adverse events during the treatment phase

	Treatment Phase Dose				TRIUMPH	
Preferred Term	32 mcg N = 2 n (%)	48 mcg N = 27 n (%)	64 mcg N = 22 n (%)	Overall N = 51 n (%)	Tyvaso n (%)	Placebo n (%)
Dyspnoea	0	2 (7.4)	1 (4.5)	3 (5.9)	6 (5)	6 (5)
Flushing	0	1 (3.7)	1 (4.5)	2 (3.9)	17 (15)	1 (<1)
Nausea	0	2 (7.4)	0	2 (3.9)	22 (19)	13 (11)
Throat irritation	0	1 (3.7)	1 (4.5)	2 (3.9)	29 (25)*	17 (14)*

*TRIUMPH groups together Throat Irritation and Pharyngolaryngeal Pain.

TABLE 20

Most frequent adverse events during the treatment phase during the optional extension phase

	TreT Dose in Treatment Phase			
Preferred Term	32 mcg N = 2 n (%)	48 mcg N = 26 n (%)	64 mcg N = 21 n (%)	Overall N = 49 n (%)
Cough	0	3 (11.5)	2 (9.5)	5 (10.2)
Dyspnoea	1 (50.0)	2 (7.7)	2 (9.5)	5 (10.2)
Headache	0	2 (7.7)	2 (9.5)	4 (8.2)
Diarrhoea	0	1 (3.8)	2 (9.5)	3 (6.1)
Pneumonia	0	2 (7.7)	1 (4.8)	3 (6.1)
Arthralgia	0	2 (7.7)	1 (4.8)	3 (6.1)
Dizziness	0	2 (7.7)	1 (4.8)	3 (6.1)

Conclusions

Transition from Tyvaso to TreT was safe and well tolerated in this study. Most adverse effects (AEs) were mild to moderate in severity and occurred at severities and frequencies consistent with those seen in other inhaled treprostinil studies in patients with PAH.

Following 3 weeks of TreT administration, subjects switching from Tyvaso to TreT demonstrated:

Significant improvements in 6MWD (8.0 m increase; p=0.0217) at Week 3. As of 23 Dec. 2020 (data cut-off date), improvements in 6MWD for TreT overall were sustained in the OEP Significant satisfaction with and preference for the use of the TreT inhaler (PQ-ITD) Significant improvement in PAH impact scores, and a trend of improvement in PAH symptom scores (PAH SYMPACT).

Additional Embodiments

1. A method of treating interstitial lung disease (ILD) or pulmonary fibrosis in a subject in need, comprising administering to the subject a therapeutically effective amount of treprostinil, a prodrug, salt, or ester thereof.

2. A method of reducing pulmonary function decline in a subject with interstitial lung disease (ILD) or pulmonary fibrosis, comprising administering to the subject treprostinil, a prodrug, salt, or ester thereof.

3. A method of increasing forced vital capacity (FVC) in a subject suffering from ILD or pulmonary fibrosis, comprising administering to the subject treprostinil, a prodrug, salt, or ester thereof.

4. The method of any one of embodiments 1-3, wherein the ILD comprises one or more of idiopathic pulmonary fibrosis (IPF), desquamative interstitial pneumonia (DIP), acute interstitial pneumonia (AIP), nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis-associated interstitial lung disease (RB-ILD), cryptogenic organizing pneumonia (COP), lymphoid interstitial pneumonia (LIP), sarcoidosis, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, antisynthetase syndrome, silicosis, asbestosis, occupational lung disease, chronic hypersensitivity pneumonitis, idiopathic interstitial pneumonia (IIP), an autoimmune ILD, lymphangioleiomyomatosis (LAM), Langerhan's cell histiocytosis (LCH), drug associated ILD, vasculitis, granulomatosis, and berylliosis.

5. The method of embodiment 4, wherein the ILD comprises IPF.

6. The method of any one of embodiments 1-5, wherein the ILD comprises systemic sclerosis-associated interstitial lung disease (SSc-ILD).

7. The method of any one of embodiments 1-6, wherein the ILD was induced from antibiotics, chemotherapy, antiarrhythmic agents, coronavirus disease 2019, atypical pneumonia, pneumocystis pneumonia, tuberculosis (TB), *Chlamydia trachomatis*, respiratory syncytial virus, or lymphangitic carcinomatosis.

8. The method of any one of embodiments 1-7, wherein the subject has one or more of surfactant-protein-B deficiency, surfactant-protein-C deficiency, ABCA3-deficiency, brain lung thyroid syndrome, congenital pulmonary alveolar proteinosis, alveolar capillary dysplasia, mutations in telomerase reverse transcriptase, mutations in telomerase RNA component, mutations in the regulator of telomere elongation helicase 1, and mutations in poly(A)-specific ribonuclease.

9. The method of any one of embodiments 1-8, wherein the subject has one or more symptoms of shortness of breath, fatigue, weight loss, dry cough, chest pain, and lung hemorrhage.

10. The method of embodiment 9, wherein after administration the symptom is improved by about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, or about 100%, as measured by a medically-recognized technique.

11. The method of embodiment 10, wherein the medically-recognized technique comprises one or more of Modified Medical Research Council (MMRC) Dyspnoea Scale, Modified Borg Dyspnoea Scale (0-10), Chalder Fatigue Scale, weight measurement scale, visual analogue scale (VAS) for cough, King's Brief Interstitial Lung Disease Questionnaire, Leicester Cough Questionnaire (LCQ), computed tomography (CT) scan, X-ray, multiple magnetic

resonance imaging (MRI), pulmonary function testing (PFT), spirometry, lung volumes, maximal respiratory pressure, diffusing capacity, oxygen desaturation, and arterial blood gas evaluation.

12. The method of any one of embodiments 1-11, wherein treprostinil, a prodrug, salt, or ester thereof is administered in a pharmaceutical composition comprising treprostinil, a prodrug, salt, or ester thereof and a pharmaceutically acceptable carrier or excipient.

13. The method of claim any one of embodiments 1-12, wherein the administration comprises at least one of oral, inhalation, subcutaneous, nasal, intravenous, intramuscular, sublingual, buccal, rectal, vaginal, and transdermal administration.

14. The method of any one of embodiments 1-13, wherein the administration comprises inhalation.

15. The method of any one of embodiments 1-14, wherein a single inhalation administration event comprises from 1 to 20 breaths.

16. The method of any one of embodiments 1-15, comprising administration of at least one additional active agent to treat the IRD.

17. The method of embodiment 16, wherein the at least one additional active agent comprises a corticosteroid, mycophenolic acid, mycophenolate mofetil, azathioprine, cyclophosphamide, rituximab, pirfenidone, or nintedanib.

18. The method of embodiment 16 or 17, wherein the at least one additional active agent and treprostinil, a prodrug, salt, or ester thereof, are administered via a method selected from the group consisting of

(a) concomitantly;

(b) as an admixture;

(c) separately and simultaneously or concurrently; and

(d) separately and sequentially.

19. The method of any one of embodiments 1-18, wherein administration is once, twice, thrice, four times, five times, or six times per day.

20. The method of any one of embodiments 1-19, wherein administration is for a period selected from the group consisting of about 1 day, about 1 day to about 3 days, about 3 days to about 6 days, about 6 days to about 9 days, about 9 days to about 12 days, about 12 days to about 15 days, about 15 days to about 18 days, about 18 days to about 21 days, about 21 days to about 24 days, about 24 days to about 27 days, about 27 days to about 30 days, or about greater than 30 days.

21. The method of any one of embodiments 1-20, wherein the subject is a human.

22. The method of any one of embodiments 1-21, wherein the method results in an increased FVC compared to the FVC at the start of or prior to the start of administration.

23. The method of embodiment 22, wherein the administration results in an increased FVC at sixteen weeks after the start of administration compared to the FVC at the start of or prior to the start of administration.

24. The method of any one of embodiments 22-23, wherein the increase in FVC is at least 20%.

25. The method of embodiment 24, wherein the increase is FVC is at least 75%.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

All of the publications, patent applications and patents cited in this specification are incorporated herein by reference in their entirety.

What is claimed is:

1. A method of improving exercise capacity in a patient having pulmonary hypertension associated with interstitial lung disease, comprising administering by inhalation to the patient having pulmonary hypertension associated with interstitial lung disease an effective amount of at least 15 micrograms up to a maximum tolerated dose of treprostinil or a pharmaceutically acceptable salt thereof in a single administration event that comprises at least 6 micrograms per breath.

2. The method of claim **1**, wherein said administering provides a statistically significant increase of a 6 minutes walk distance in the patient after 8 weeks, 12 weeks, or 16 weeks of the administering.

3. The method of claim **1**, wherein said administering increases a 6 minutes walk distance of the patient by at least 10 m after 8 weeks, 12 weeks, or 16 weeks of the administering.

4. The method of claim **1**, wherein said administering provides a statistically significant reduction of a plasma concentration of NT-proBNP in the patient after 8 weeks, 12 weeks, or 16 weeks of the administering.

5. The method of claim **1**, wherein said administering reduces a plasma concentration of NT-proBNP in the patient by at least 200 pg/ml after 8 weeks, 12 weeks, or 16 weeks of the administering.

6. The method of claim **1**, wherein said administering provides a statistically significant reduction of at least one exacerbations of the interstitial lung disease.

7. The method of claim **1**, wherein said administering provides a statistically significant reduction of clinical worsening events due to the interstitial lung disease.

8. The method of claim **7**, wherein the clinical worsening events comprise at least one of hospitalization for cardiopulmonary indication and a decrease in a 6-minute walk distance by more than 15% compared a baseline 6-minute walk distance prior to the administering.

9. The method of claim **1**, wherein said administering provides a statistically significant improves of forced vital capacity (FVC) in the patient after 8 weeks, 12, weeks or 16 weeks of the administering.

10. The method of claim **9**, wherein said administering improves the forced vital capacity (FVC) in the patient by at least 20 ml after 8 weeks, 12 weeks, or 16 weeks of the administering.

11. The method of claim **1**, wherein said administering is performed by a pulsed inhalation device.

12. The method of claim **11**, wherein the pulsed inhalation device contains an inhalation solution comprising treprostinil or a pharmaceutically acceptable salt thereof.

13. The method of claim **11**, wherein the pulsed inhalation device is a nebulizer.

14. The method of claim **11**, wherein the pulsed inhalation device is a dry powder inhaler comprising a dry powder comprising treprostinil or a pharmaceutically acceptable salt thereof.

15. The method of claim **1**, wherein the effective amount of treprostinil or a pharmaceutically acceptable salt administered to the patient in a single inhalation administration event is from 15 μg to 100 μg.

16. The method of claim **15**, wherein the single inhalation administration event does not exceed 15 breaths by the patient.

17. The method of claim 1, wherein said administering increases a 6 minutes walk distance of the patient by at least 10 m after 8 weeks of the administering.

18. The method of claim 1, wherein said administering increases a 6 minutes walk distance of the patient by at least 15 m after 12 weeks of the administering.

19. The method of claim 1, wherein said administering increases a 6 minutes walk distance of the patient by at least 15 m after 16 weeks of the administering.

* * * * *

EXHIBIT H

US010716793B2

(12) **United States Patent**
Olschewski et al.

(10) **Patent No.: US 10,716,793 B2**
(45) **Date of Patent: *Jul. 21, 2020**

(54) **TREPROSTINIL ADMINISTRATION BY INHALATION**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(60) Continuation of application No. 16/536,954, filed on Aug. 9, 2019, which is a continuation of application No. 15/011,999, filed on Feb. 1, 2016, now Pat. No. 10,376,525, which is a division of application No. 13/469,854, filed on May 11, 2012, now Pat. No. 9,339,507, which is a division of application No. 12/591,200, filed on Nov. 12, 2009, now Pat. No. 9,358,240, which is a continuation of application No. 11/748,205, filed on May 14, 2007, now abandoned.

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Treprostinil can be administered using a metered dose inhaler. Such administration provides a greater degree of autonomy to patients. Also disclosed are kits that include a metered dose inhaler containing a pharmaceutical formulation containing treprostinil.

8 Claims, 12 Drawing Sheets

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FIGURE 1

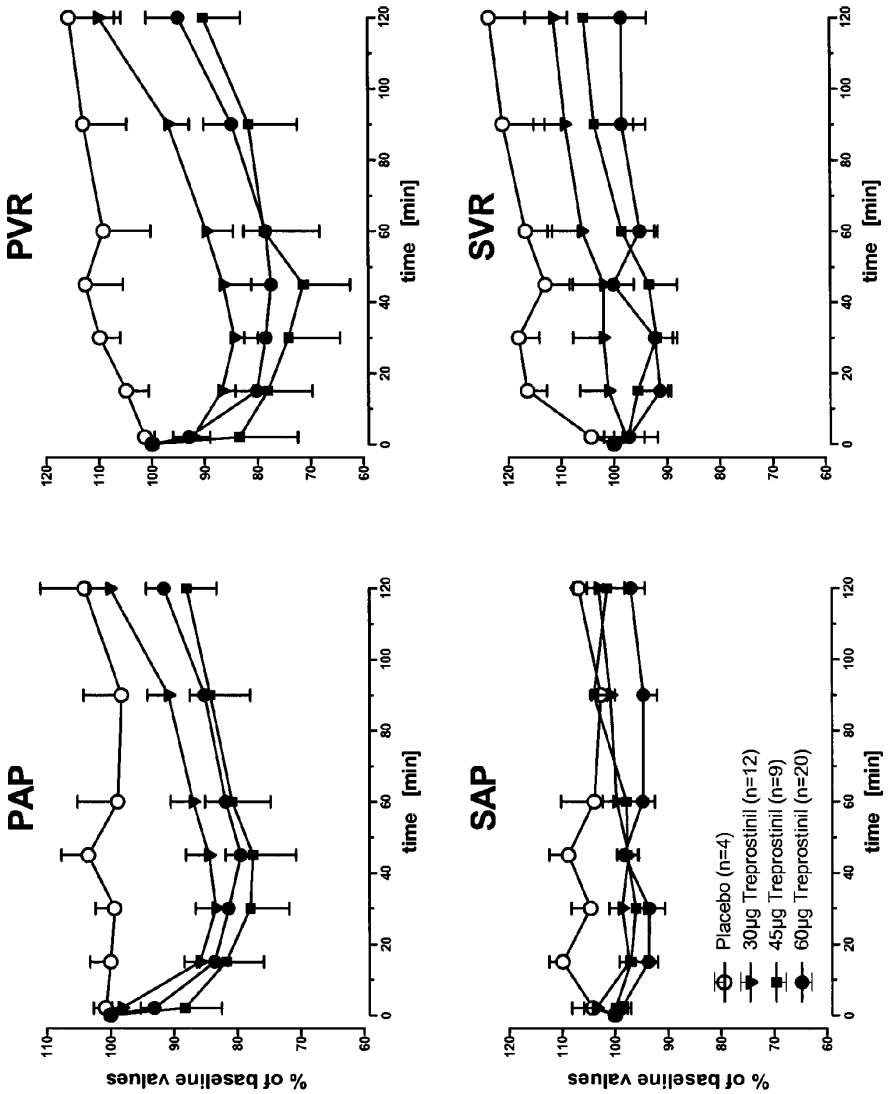

PVR
SVR
PAP
SAP
time [min]
% of baseline values
Placebo (n=4)
30µg Treprostinil (n=12)
45µg Treprostinil (n=9)
60µg Treprostinil (n=20)

FIGURE 2

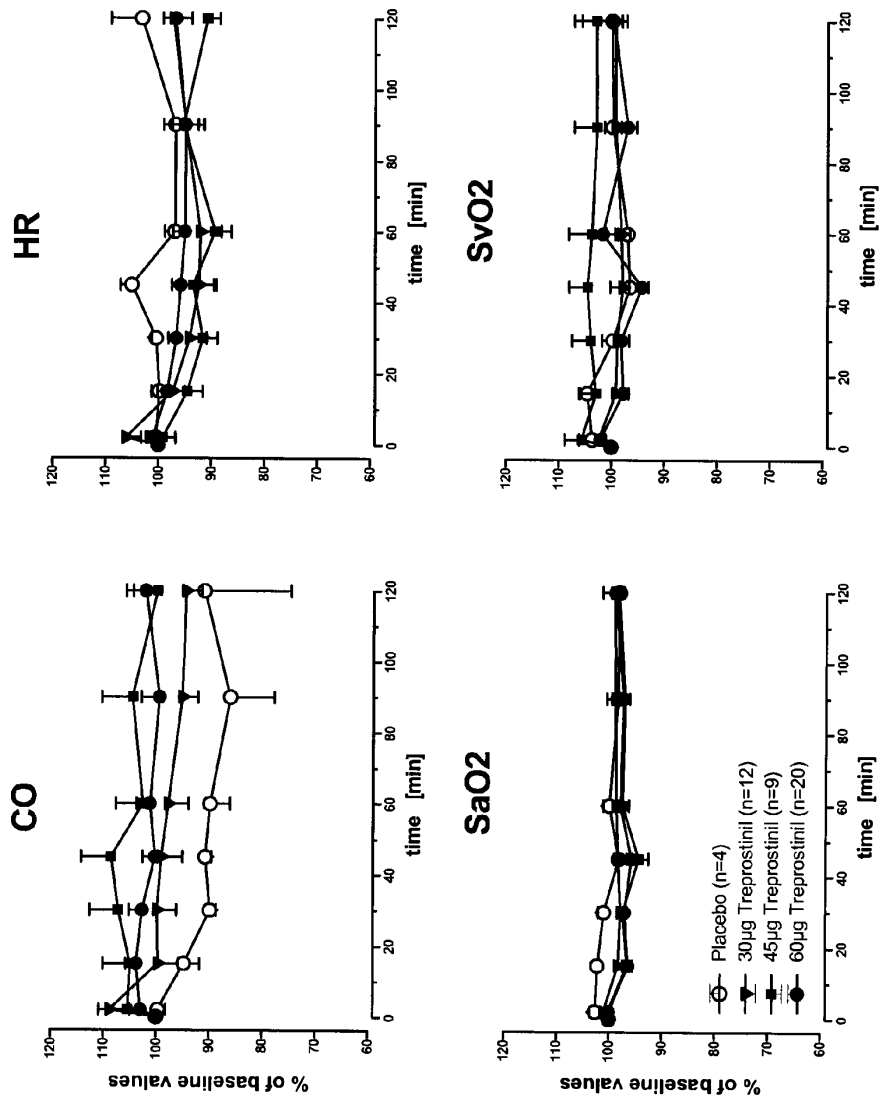

HR
SvO2
CO
SaO2
time [min]
% of baseline values
Placebo (n=4)
30µg Treprostinil (n=12)
45µg Treprostinil (n=9)
60µg Treprostinil (n=20)

FIGURE 3

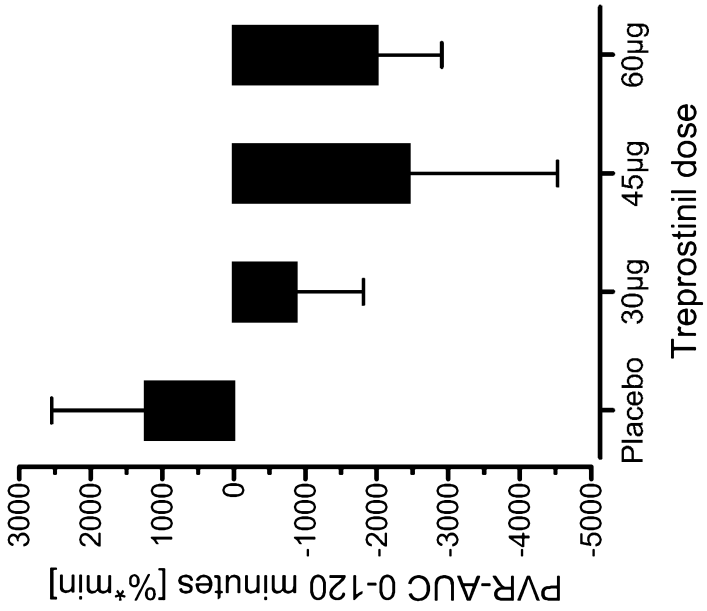

PVR-AUC 0-120 minutes [%*min]
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
Placebo
30µg
45µg
60µg
Treprostinil dose

FIGURE 4

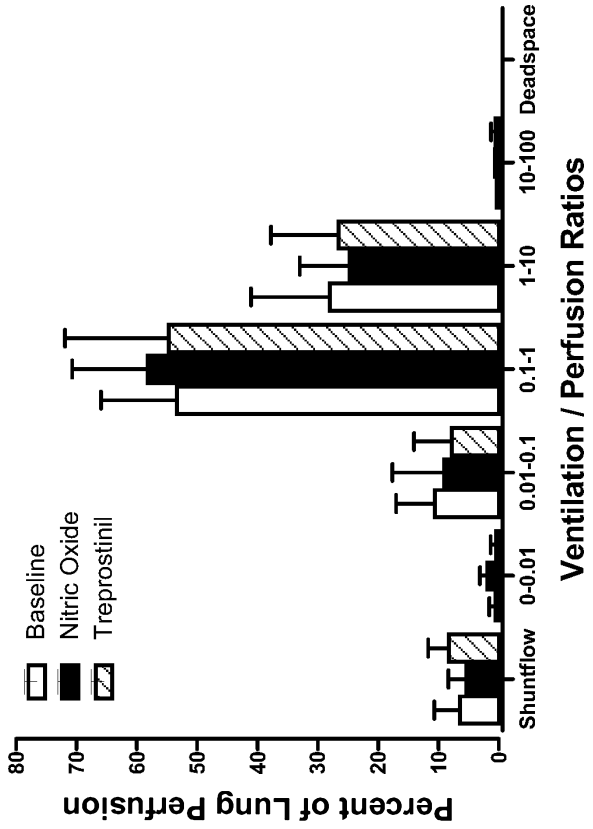

Percent of Lung Perfusion
0
10
20
30
40
50
60
70
80
Baseline
Nitric Oxide
Treprostinil
Shuntflow
0-0.01
0.01-0.1
0.1-1
1-10
10-100
Deadspace
Ventilation / Perfusion Ratios

FIGURE 5

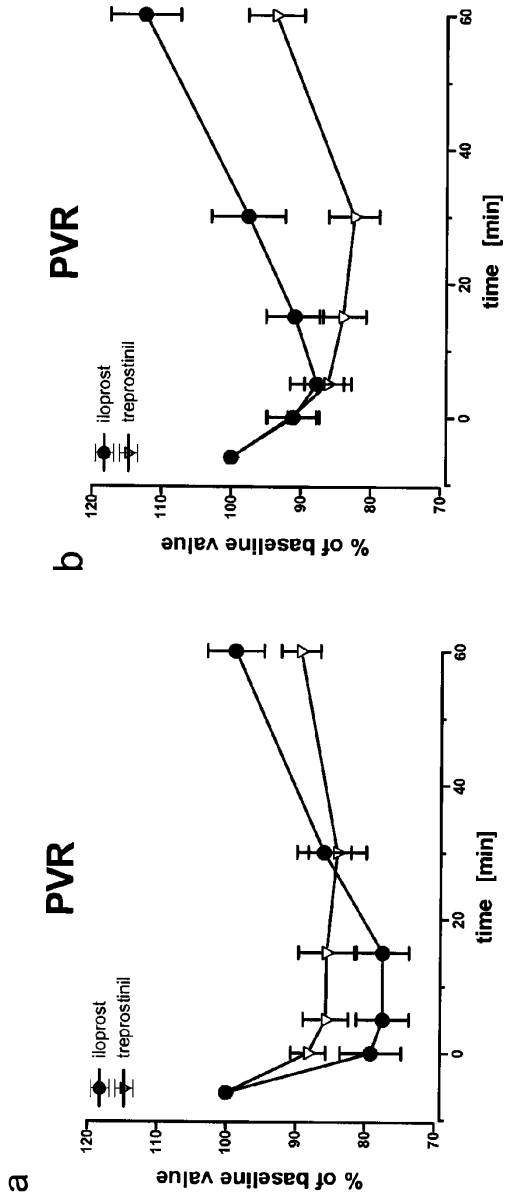

a
PVR
iloprost
treprostinil
% of baseline value
120
110
100
90
80
70
0
20
40
60
time [min]
b
PVR
iloprost
treprostinil
% of baseline value
120
110
100
90
80
70
0
20
40
60
time [min]

FIGURE 6

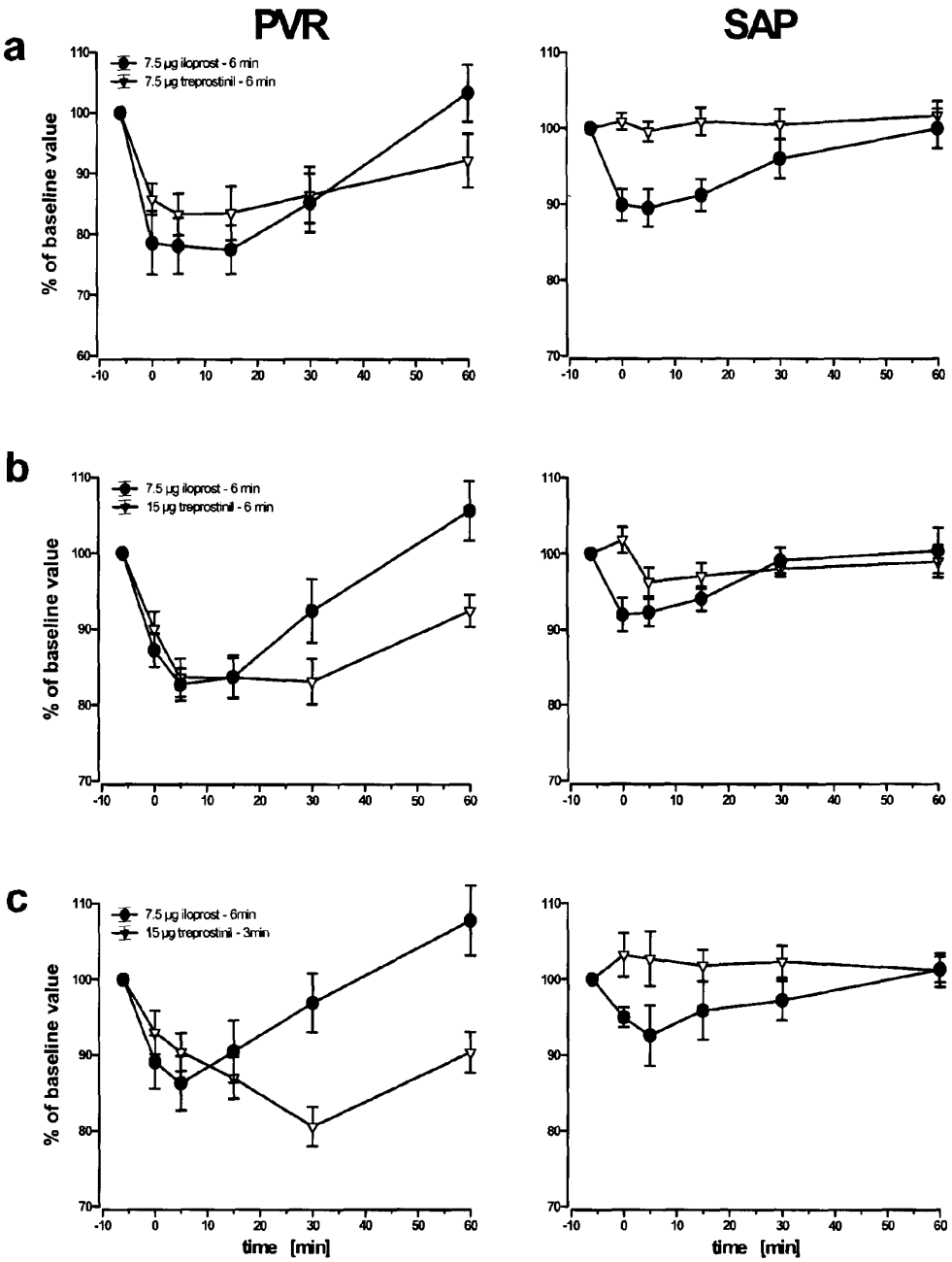

a
PVR
SAP
7.5 µg iloprost - 6 min
7.5 µg treprostinil - 6 min
b
7.5 µg iloprost - 6 min
15 µg treprostinil - 6 min
c
7.5 µg iloprost - 6min
15 µg treprostinil - 3min
% of baseline value
time [min]

FIGURE 7

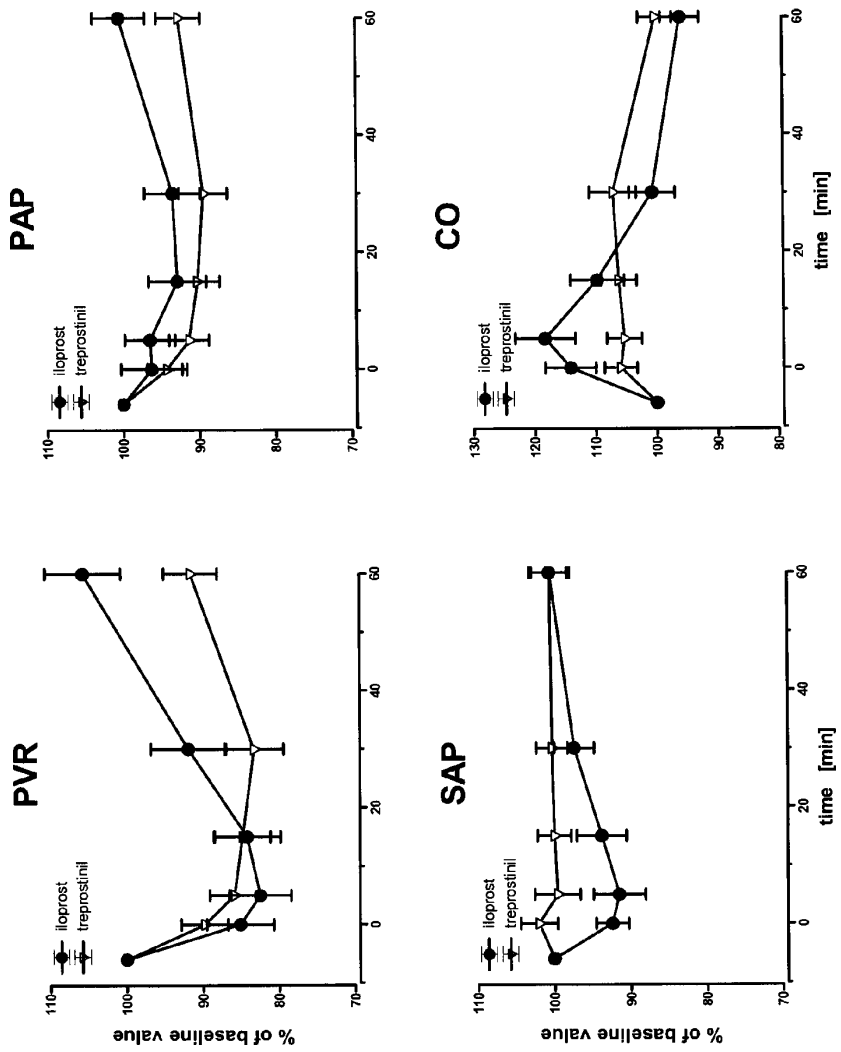

PAP
CO
PVR
SAP
iloprost
treprostinil
% of baseline value
time [min]

FIGURE 8

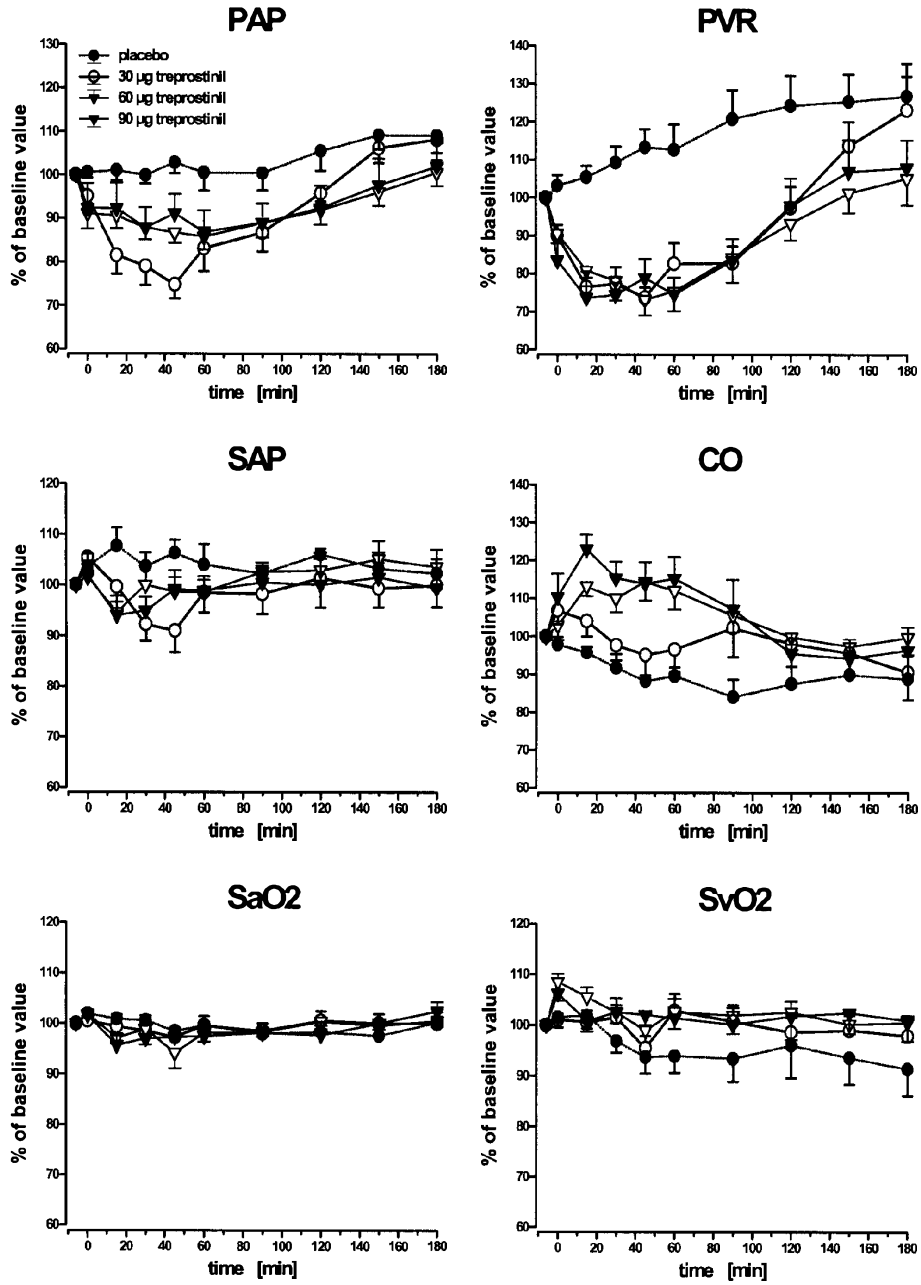

PAP
PVR
SAP
CO
SaO2
SvO2
placebo
30 µg treprostinil
60 µg treprostinil
90 µg treprostinil
% of baseline value
time [min]

FIGURE 9

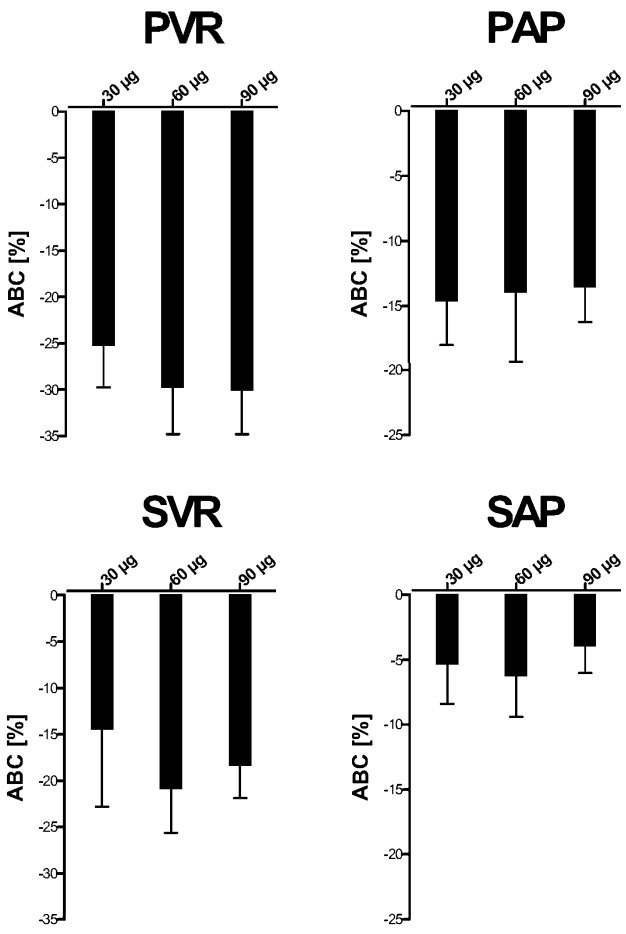


FIGURE 10

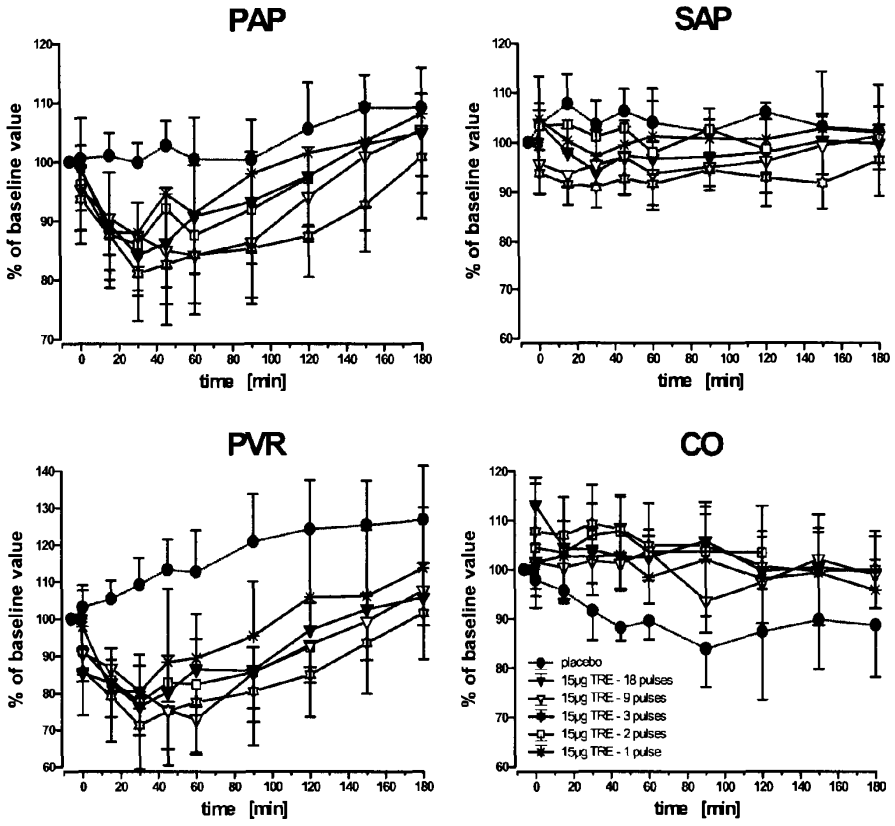

PAP
SAP
PVR
CO
% of baseline value
time [min]
placebo
15µg TRE - 18 pulses
15µg TRE - 9 pulses
15µg TRE - 3 pulses
15µg TRE - 2 pulses
15µg TRE - 1 pulse

FIGURE 11

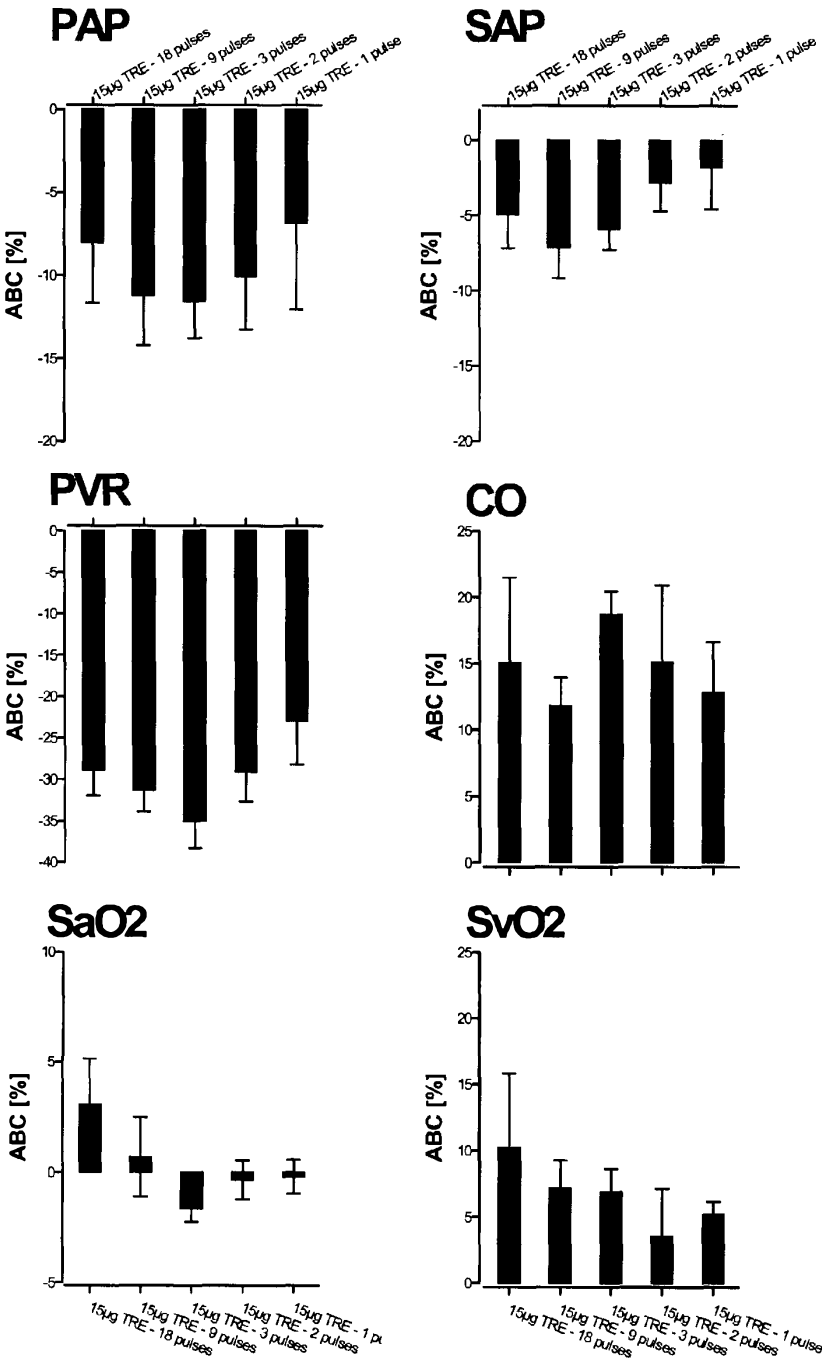
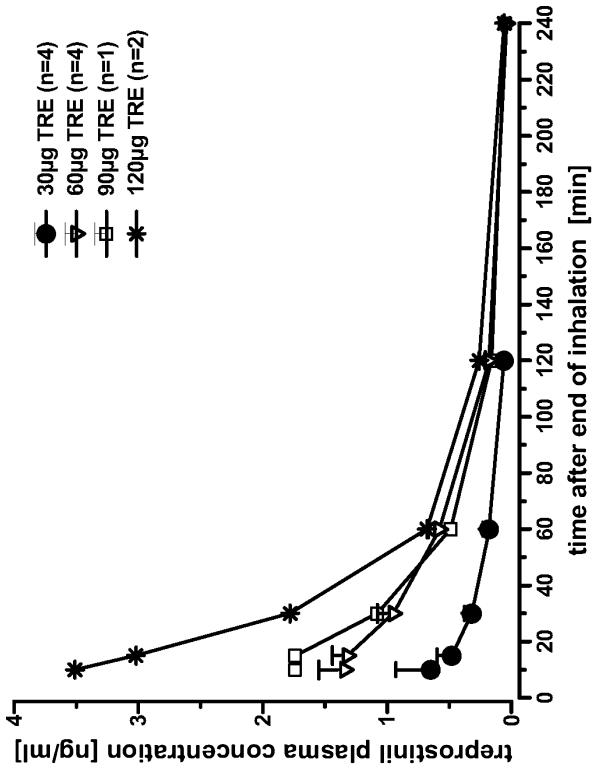

PAP
SAP
PVR
CO
SaO2
SvO2
ABC [%]
15µg TRE - 18 pulses
15µg TRE - 9 pulses
15µg TRE - 3 pulses
15µg TRE - 2 pulses
15µg TRE - 1 pulse

FIGURE 12


30µg TRE (n=4)
60µg TRE (n=4)
90µg TRE (n=1)
120µg TRE (n=2)
treprostinil plasma concentration [ng/ml]
0
1
2
3
4
time after end of inhalation [min]
0
20
40
60
80
100
120
140
160
180
200
220
240

TREPROSTINIL ADMINISTRATION BY INHALATION

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is a Continuation of U.S. application Ser. No. 16/536,954, filed Aug. 9, 2019, which is a Continuation of U.S. application Ser. No. 15/011,999, filed Feb. 1, 2016, which is a Divisional of U.S. application Ser. No. 13/469,854, filed May 11, 2012, Divisional of U.S. application Ser. No. 12/591,200, filed Nov. 12, 2009, which is a Continuation of U.S. application Ser. No. 11/748,205, filed May 14, 2007, which claims priority to U.S. provisional application No. 60/800,016 filed May 15, 2006, which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

The present application relates to methods and kits for therapeutic treatment and, more particularly, to therapeutic methods involving administering treprostinil using a metered dose inhaler and related kits.

BACKGROUND OF THE INVENTION

All blood is driven through the lungs via the pulmonary circulation in order, among other things, to replenish the oxygen which it dispenses in its passage around the rest of the body via the systemic circulation. The flow through both circulations is in normal circumstances equal, but the resistance offered to it in the pulmonary circulation is generally much less than that of the systemic circulation. When the resistance to pulmonary blood flow increases, the pressure in the circulation is greater for any particular flow. The above described condition is referred to as pulmonary hypertension (PH). Generally, pulmonary hypertension is defined through observations of pressures above the normal range pertaining in the majority of people residing at the same altitude and engaged in similar activities.

Pulmonary hypertension may occur due to various reasons and the different entities of pulmonary hypertension were classified based on clinical and pathological grounds in 5 categories according to the latest WHO convention, see e.g. Simonneau G., et al. J. Am. Coll. Cardiol. 2004; 43(12 Suppl S):5S-12S. Pulmonary hypertension can be a manifestation of an obvious or explicable increase in resistance, such as obstruction to blood flow by pulmonary emboli, malfunction of the heart's valves or muscle in handling blood after its passage through the lungs, diminution in pulmonary vessel caliber as a reflex response to alveolar hypoxia due to lung diseases or high altitude, or a mismatch of vascular capacity and essential blood flow, such as shunting of blood in congenital abnormalities or surgical removal of lung tissue. In addition, certain infectious diseases, such as HIV and liver diseases with portal hypertension may cause pulmonary hypertension. Autoimmune disorders, such as collagen vascular diseases, also often lead to pulmonary vascular narrowing and contribute to a significant number of pulmonary hypertension patients. The cases of pulmonary hypertension remain where the cause of the increased resistance is as yet inexplicable are defined as idiopathic (primary) pulmonary hypertension (iPAH) and are diagnosed by and after exclusion of the causes of secondary pulmonary hypertension and are in the majority of cases related to a genetic mutation in the bone morphogenetic protein receptor-2 gene. The cases of idiopathic pulmonary arterial hypertension tend to comprise a recognizable entity of about 40% of patients cared for in large specialized pulmonary hypertension centers. Approximately 65% of the most commonly afflicted are female and young adults, though it has occurred in children and patients over 50. Life expectancy from the time of diagnosis is short without specific treatment, about 3 to 5 years, though occasional reports of spontaneous remission and longer survival are to be expected given the nature of the diagnostic process. Generally, however, disease progress is inexorable via syncope and right heart failure and death is quite often sudden.

Pulmonary hypertension refers to a condition associated with an elevation of pulmonary arterial pressure (PAP) over normal levels. In humans, a typical mean PAP is approximately 12-15 mm Hg. Pulmonary hypertension, on the other hand, can be defined as mean PAP above 25 mmHg, assessed by right heart catheter measurement. Pulmonary arterial pressure may reach systemic pressure levels or even exceed these in severe forms of pulmonary hypertension. When the PAP markedly increases due to pulmonary venous congestion, i.e. in left heart failure or valve dysfunction, plasma can escape from the capillaries into the lung interstitium and alveoli. Fluid buildup in the lung (pulmonary edema) can result, with an associated decrease in lung function that can in some cases be fatal. Pulmonary edema, however, is not a feature of even severe pulmonary hypertension due to pulmonary vascular changes in all other entities of this disease.

Pulmonary hypertension may either be acute or chronic. Acute pulmonary hypertension is often a potentially reversible phenomenon generally attributable to constriction of the smooth muscle of the pulmonary blood vessels, which may be triggered by such conditions as hypoxia (as in high-altitude sickness), acidosis, inflammation, or pulmonary embolism. Chronic pulmonary hypertension is characterized by major structural changes in the pulmonary vasculature, which result in a decreased cross-sectional area of the pulmonary blood vessels. This may be caused by, for example, chronic hypoxia, thromboembolism, collagen vascular diseases, pulmonary hypercirculation due to left-to-right shunt, HIV infection, portal hypertension or a combination of genetic mutation and unknown causes as in idiopathic pulmonary arterial hypertension.

Pulmonary hypertension has been implicated in several life-threatening clinical conditions, such as adult respiratory distress syndrome ("ARDS") and persistent pulmonary hypertension of the newborn ("PPHN"). Zapol et al., Acute Respiratory Failure, p. 241-273, Marcel Dekker, New York (1985); Peckham, J. Ped. 93:1005 (1978). PPHN, a disorder that primarily affects full-term infants, is characterized by elevated pulmonary vascular resistance, pulmonary arterial hypertension, and right-to-left shunting of blood through the patent ductus arteriosus and foramen ovale of the newborn's heart. Mortality rates range from 12-50%. Fox, Pediatrics 59:205 (1977); Dworetz, Pediatrics 84:1 (1989). Pulmonary hypertension may also ultimately result in a potentially fatal heart condition known as "cor pulmonale," or pulmonary heart disease. Fishman, "Pulmonary Diseases and Disorders" 2nd Ed., McGraw-Hill, New York (1988).

Currently, there is no treatment for pulmonary hypertension that can be administered using a compact inhalation device, such as a metered dose inhaler.

SUMMARY OF THE INVENTION

One embodiment is a method of delivering to a subject in need thereof a therapeutically effective amount of trepros-

tinil, or treprostinil derivative or a pharmaceutically acceptable salt thereof comprising administering to the subject a therapeutically effective amount of the treprostinil or treprostinil derivative or a pharmaceutically acceptable salt thereof using a metered dose inhaler.

Another embodiment is a method for treating pulmonary hypertension comprising administering to a subject in need thereof treprostinil or its derivative, or a pharmaceutically acceptable salt thereof using a metered dose inhaler.

Yet another embodiment is a kit comprising a metered dose inhaler containing a pharmaceutical formulation comprising treprostinil or treprostinil derivative, or a pharmaceutically acceptable salt thereof.

And yet another embodiment is a kit for treating pulmonary hypertension in a subject, comprising (i) an effective amount of treprostinil or its derivative, or a pharmaceutically acceptable salt thereof; (ii) a metered dose inhaler; (iii) instructions for use in treating pulmonary hypertension.

Administration of treprostinil using a metered dose inhaler can provide patients, such as pulmonary hypertension patients, with a high degree of autonomy.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** pulmonary and systemic changes in hemodynamics following the inhalation of placebo (open circles), 30 μg treprostinil (triangles), 45 μg treprostinil (squares) or 60 μg TREprostinil (black circles) applied by a Metered Dose Inhaler (MDI-TRE). A single short inhalation of treprostinil induced sustained reduction of PAP and PVR that outlasted the observation period of 120 minutes at doses of 45 and 60 μg MDI-TRE. Systemic arterial pressure and resistance were not significantly affected. PAP=mean pulmonary artery pressure; PVR=pulmonary vascular resistance; SAP=mean systemic arterial pressure; SVR=systemic vascular resistance. Data are given as mean value±standard error of the mean (SEM).

FIG. **2** presents hemodynamic changes induced by the inhalation of placebo (open circles), 30 μg treprostinil (triangles), 45 μg treprostinil (squares) or 60 μg treprostinil (black circles) applied by a metered dose inhaler. Treprostinil induced sustained elevation of cardiac output. Heart rate was rather unchanged as a sign for low spillover of MDI-TRE to the systemic circulation. Gas exchange was not negatively affected. CO=cardiac output; HR=heart rate; SaO2=arterial oxygen saturation; SvO2=central venous oxygen saturation. Data are given as mean value±SEM.

FIG. 3 shows areas under the curve for changes in pulmonary vascular resistance (PVR) calculated for an observation period of 120 minutes after inhalation treprostinil using a metered dose inhaler. PVR was markedly lowered by treprostinil inhalation. The increased pulmonary vasodilation over time with the two highest doses mainly relies on the more sustained effect over time. Data are shown as mean value±95% confidence intervals.

FIG. **4** demonstrates Ventilation-perfusion matching measured with the multiple inert gas elimination technique. Five patients (30 μg TRE, n=2; 45 μg TRE, n=1; 60 μg TRE, n=2) with pre-existing gas exchange problems were investigated for changes in ventilation-perfusion ratios. All patients had significant shunt flow at baseline. Shunt-flow and low V/Q areas were not significantly changed by nitric oxide (NO) inhalation or treprostinil inhalation using a metered dose inhaler (MDI-TRE). MDI-TRE applied at high treprostinil concentrations did not negatively affect ventilation-perfusion matching and gas-exchange. Data are given as mean value±95% confidence intervals.

FIG. **5** presents response of pulmonary vascular resistance (PVR) to inhaled treprostinil vs. iloprost—period effects. a) First inhalation with treprostinil (n=22) vs. first inhalation with iloprost (n=22); b) second inhalation with treprostinil (n=22) vs. second inhalation with iloprost (n=22). The PVR decrease with treprostinil was delayed and prolonged, compared to iloprost. Due to carryover effects from the first period, in the second period, the effects of both drugs appeared shortened. Data are shown as percent of baseline values (mean value±95% confidence interval).

FIG. **6** presents response of PVR and systemic arterial pressure (SAP) to inhalation of treprostinil vs. iloprost—dose effects. a) Inhalation of 7.5 μg iloprost (in 6 min) vs. 7.5 μg treprostinil (6 min) (n=14, in a randomized order). b) Inhalation of 7.5 μg iloprost (6 min) vs. 15 μg treprostinil (6 min) (n=14, in randomized order). c) Inhalation of 7.5 μg iloprost (6 min) vs. 15 μg treprostinil (3 min) (n=16, in randomized order). Data are shown as percent of baseline values (mean±95% confidence interval). Iloprost, filled circles; Treprostinil, open triangles.

FIG. **7** presents hemodynamic response to inhalation of treprostinil vs. iloprost. Data from n=44 patients, who inhaled both drugs in randomized order, shown as percent of baseline values (mean value±95% confidence interval). PVR, pulmonary vascular resistance; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure; CO, cardiac output.

FIG. **8** presents pharmacodynamics after treprostinil inhalation vs. placebo. Placebo or treprostinil in doses of 30 μg, 60 μg or 90 μg were inhaled (means±95% confidence intervals). Maximal decrease of PVR was comparable for all doses. The duration of pulmonary vasodilation (PVR-decrease) appeared to be dose dependent. PVR, pulmonary vascular resistance; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure; CO, cardiac output; SaO2, arterial oxygen saturation; SvO2, mixed venous oxygen saturation.

FIG. **9** presents Areas Between the placebo and the treprostinil Curves (ABC). ABCs were calculated for a 3-hour period after inhalation of TRE or placebo from the relative changes of hemodynamic parameters (means±95% confidence intervals). PVR, pulmonary vascular resistance; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure; SVR, systemic vascular resistance.

FIG. **10** presents hemodynamic responses to the inhalation of 15 μg treprostinil. The inhalation time by increasing treprostinil concentration. A pulse of aerosol was generated every 6 seconds. TRE aerosol was inhaled in concentrations of 100 μg/ml (18 pulses; n=6), 200 μg/ml (9 pulses; n=6), 600 μg/ml (3 pulses; n=21), 1000 μg/ml (2 pulses; n=7) and 2000 μg/ml (1 pulse; n=8). Placebo data correspond to FIG. **8**. Data are shown as means±95% confidence intervals. PVR, pulmonary vascular resistance; PAP, mean pulmonary arterial pressure; SAP, mean systemic arterial pressure; CO, cardiac output.

FIG. **11** presents areas between the placebo curve and the responses to 15 μg treprostinil applied at increasing concentrations to minimize inhalation time. Mean±SEM of relative changes of hemodynamic parameters (observation time 120 min). PAP, pulmonary arterial pressure, SAP, systemic arterial pressure, PVR, pulmonary vascular resistance, CO, cardiac output, SaO2, systemic arterial oxygen saturation, SvO2, pulmonary arterial oxygen saturation.

FIG. **12** presents pharmacokinetics of treprostinil after a single inhalation. Treprostinil plasma levels after inhalation of 30 μg, 60 μg, 90 μg or 120 μg treprostinil (6 min

inhalation period; experiments correspond to those shown in FIGS. **8** and **9**). Data with error bars represent mean values±SEM.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise specified, the term "a" or "an" used herein shall mean "one or more."

The present application incorporates herein by reference in its entirety Voswinckel R, et al. J. Am. Coll. Cardiol. 2006; 48:1672-1681.

The inventors discovered that a therapeutically effective dose of treprostinil can be administered in a few single inhalations using a compact inhalation device, such as a metered dose inhaler. Furthermore, the inventors discovered that such administering does not cause significant side effects, especially no significant side effects related to systemic blood pressure and circulation as well as no gas exchange deteriorations or disruptions.

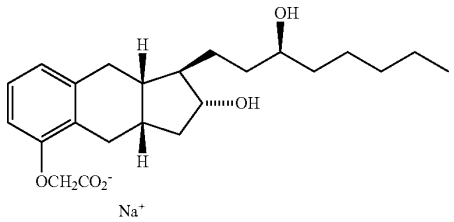
Accordingly, one embodiment of the invention is a method of delivering to a subject in need thereof, such as a human being, a therapeutically effective amount of treprostinil comprising administering to the subject a formulation comprising a therapeutically effective amount of treprostinil, its derivative or a pharmaceutically acceptable salt thereof using a metered dose inhaler. Treprostinil can be administered via a metered dose inhaler to a subject affected with a condition or disease, which can be treated by treprostinil, such as asthma, pulmonary hypertension, peripheral vascular disease or pulmonary fibrosis.

Another embodiment of the invention is a method for treating pulmonary hypertension, comprising administering to a subject in need thereof, such as a human being, treprostinil or its derivative, or a pharmaceutically acceptable salt using a metered dose inhaler.

Treprostinil, or 9-deoxy-2',9-alpha-methano-3-oxa-4,5,6-trinor-3,7-(1'3'-interphenylene)-13,14-dihydro-prostaglandin F1, is a prostacyclin analogue, first described in U.S. Pat. No. 4,306,075. U.S. Pat. No. 5,153,222 describes use of treprostinil for treatment of pulmonary hypertension. Treprostinil is approved for the intravenous as well as subcutaneous route, the latter avoiding septic events associated with continuous intravenous catheters. U.S. Pat. Nos. 6,521,212 and 6,756,033 describe administration of treprostinil by inhalation for treatment of pulmonary hypertension, peripheral vascular disease and other diseases and conditions. U.S. Pat. No. 6,803,386 discloses administration of treprostinil for treating cancer such as lung, liver, brain, pancreatic, kidney, prostate, breast, colon and head-neck cancer. US patent application publication No. 2005/0165111 discloses treprostinil treatment of ischemic lesions. U.S. Pat. No. 7,199,157 discloses that treprostinil treatment improves kidney functions. US patent application publication No. 2005/0282903 discloses treprostinil treatment of neuropathic foot ulcers. U.S. provisional application No. 60/900,320 filed Feb. 9, 2007, discloses treprostinil treatment of pulmonary fibrosis.

The term "acid derivative" is used herein to describe C1-4 alkyl esters and amides, including amides wherein the nitrogen is optionally substituted by one or two C1-4 alkyl groups.

The present invention also encompasses methods of using Treprostinil or its derivatives, or pharmaceutically acceptable salts thereof. In one embodiment, a method uses Treprostinil sodium, currently marketed under the trade name of REMODULIN®. The FDA has approved Treprostinil sodium for the treatment of pulmonary arterial hypertension by injection of dose concentrations of 1.0 mg/mL, 2.5 mg/mL, 5.0 mg/mL and 10.0 mg/mL. The chemical structure formula for Treprostinil sodium is:



Treprostinil sodium is sometimes designated by the chemical names: (a) [(1R,2R,3aS,9aS)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]acetic acid; or (b) 9-deoxy-2',9-α-methano-3-oxa-4,5,6-trinor-3,7-(1',3'-interphenylene)-13,14-dihydro-prostaglandin F_1. Treprostinil sodium is also known as: UT-15; LRX-15; 15AU81; UNIPROST™; BW A15AU; and U-62,840. The molecular weight of Treprostinil sodium is 390.52, and its empirical formula is $C_{23}H_{34}O_5$.

In certain embodiments, treprostinil can be administered in combination with one or more additional active agents. In some embodiments, such one or more additional active agents can be also administered together with treprostinil using a metered dose inhaler. Yet in some embodiments, such one or more additional active agents can be administered separately from treprostinil. Particular additional active agents that can be administered in combination with treprostinil may depend on a particular disease or condition for treatment or prevention of which treprostinil is administered. In some cases, the additional active agent can be a cardiovascular agent such as a calcium channel blocker, a phosphodiesterase inhibitor, an endothelial antagonist, or an antiplatelet agent.

The present invention extends to methods of using physiologically acceptable salts of Treprostinil, as well as non-physiologically acceptable salts of Treprostinil that may be used in the preparation of the pharmacologically active compounds of the invention.

The term "pharmaceutically acceptable salt" refers to a salt of Treprostinil with an inorganic base, organic base, inorganic acid, organic acid, or basic or acidic amino acid. Salts of inorganic bases can be, for example, salts of alkali metals such as sodium or potassium; alkaline earth metals such as calcium and magnesium or aluminum; and ammonia. Salts of organic bases can be, for example, salts trimethylamine, triethylamine, pyridine, picoline, ethanolamine, diethanolamine, and triethanolamine. Salts of inorganic acids can be, for example, salts of hydrochloric acid, hydroboric acid, nitric acid, sulfuric acid, and phosphoric acid. Salts of organic acids can be, for example, salts of formic acid, acetic acid, trifluoroacetic acid, fumaric acid, oxalic acid, lactic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid. Salts of basic amino acids can be, for example, salts of arginine, lysine and ornithine. Salts of acidic amino acids can include, for example, salts of aspartic acid and glutamic acid. Quaternary ammonium salts can be formed, for example, by reaction with lower alkyl halides, such as methyl, ethyl, propyl, and butyl chlorides, bromides, and iodides, with dialkyl sul-

phates, with long chain halides, such as decyl, lauryl, myristyl, and stearyl chlorides, bromides, and iodides, and with aralkyl halides, such as benzyl and phenethyl bromides.

Preferred pharmaceutically acceptable salts are disclosed, for example, in US patent application publication No. 20050085540.

Treprostinil can be administered by inhalation, which in the present context refers to the delivery of the active ingredient or a combination of active ingredients through a respiratory passage, wherein the subject in need of the active ingredient(s) through the subject's airways, such as the subject's nose or mouth.

A metered dose inhaler in the present context means a device capable of delivering a metered or bolus dose of respiratory drug, such as treprostinil, to the lungs. One example of the inhalation device can be a pressurized metered dose inhaler, a device which produces the aerosol clouds for inhalation from solutions and/or suspensions of respiratory drugs in chlorofluorocarbon (CFC) and/or hydrofluoroalkane (HFA) solutions.

The inhalation device can be also a dry powder inhaler. In such case, the respiratory drug is inhaled in solid formulation, usually in the form of a powder with particle size less than 10 micrometers in diameter or less than 5 micrometers in diameter.

The metered dose inhaler can be a soft mist inhaler (SMI), in which the aerosol cloud containing a respiratory drug can be generated by passing a solution containing the respiratory drug through a nozzle or series of nozzles. The aerosol generation can be achieved in SMI, for example, by mechanical, electromechanical or thermomechanical process. Examples of soft mist inhalers include the Respimat® Inhaler (Boeringer Ingelheim GmbH), the AERx® Inhaler (Aradigm Corp.), the Mystic™ Inhaler (Ventaira Pharmaceuticals, Inc) and the Aira™ Inhaler (Chrysalis Technologies Incorporated). For a review of soft mist inhaler technology, see e.g. M. Hindle, The Drug Delivery Companies Report, Autumn/Winter 2004, pp. 31-34. The aerosol for SMI can be generated from a solution of the respiratory drug further containing pharmaceutically acceptable excipients. In the present case, the respiratory drug is treprostinil, its derivative or a pharmaceutically acceptable salt thereof, which can be formulated in SMI is as a solution. The solution can be, for example, a solution of treprostinil in water, ethanol or a mixture thereof. Preferably, the diameter of the treprostinil-containing aerosol particles is less than about 10 microns, or less than about 5 microns, or less than about 4 microns.

Treprostinil concentration in an aerosolable formulation, such as a solution, used in a metered dose inhaler can range from about 500 μg/ml to about 2500 μg/ml, or from about 800 μg/ml to about 2200 μg/ml, or from about 1000 μg/ml to about 2000 μg/ml.

The dose of treprostinil that can be administered using a metered dose inhaler in a single event can be from about 15 μg to about 100 μg or from about 15 μg to about 90 μg or from about 30 μg to about 90 μg or from about 30 μg to about 60 μg.

Administering of treprostinil in a single event can be carried out in a limited number of breaths by a patient. For example, treprostinil can be administered in 20 breaths or less, or in 10 breaths or less, or than 5 breaths or less. Preferably, treprostinil is administered in 3, 2 or 1 breaths.

The total time of a single administering event can be less than 5 minutes, or less than 1 minute, or less than 30 seconds.

Treprostinil can be administered a single time per day or several times per day.

In some embodiments, the method of treatment of pulmonary hypertension can further comprise administering at least one supplementary agent selected from the group consisting of sildenafil, tadalafil, calcium channel blockers (diltiazem, amlodipine, nifedipine), bosentan, sitaxsentan, ambrisentan, and pharmaceutically acceptable salts thereof. In some embodiments, the supplementary agents can be included in the treprostinil formulation and, thus, can be administered simultaneously with treprostinil using a metered dose inhaler. In some embodiments, the supplementary agents can be administered separately from treprostinil. In some embodiments, the application of intravenous prostacyclin (flolan), intravenous iloprost or intravenous or subcutaneous treprostinil can be administered in addition to treprostinil administered via inhalation using a metered dose inhaler.

The present invention also provides a kit that includes a metered dose inhaler containing a pharmaceutical formulation comprising treprostinil or its derivative, or a pharmaceutically acceptable salt thereof. Such a kit can further include instructions on how to use the metered dose inhaler for inhaling treprostinil. Such instructions can include, for example, information on how to coordinate patient's breathing, and actuation of the inhaler. The kit can be used by a subject, such as human being, affected with a disease or condition that can be treated by treprostinil, such as asthma, pulmonary hypertension, peripheral vascular disease or pulmonary fibrosis.

In some cases, the kit is a kit for treating pulmonary hypertension, that includes (i) a metered dose inhaler containing a pharmaceutical formulation comprising treprostinil or its derivative, or a pharmaceutically acceptable salt thereof; and (ii) instructions for use of the metered dose inhaler containing treprostinil in treating pulmonary hypertension.

As used herein, the phrase "instructions for use" shall mean any FDA-mandated labeling, instructions, or package inserts that relate to the administration of Treprostinil or its derivatives, or pharmaceutically acceptable salts thereof, for treatment of pulmonary hypertension by inhalation. For example, instructions for use may include, but are not limited to, indications for pulmonary hypertension, identification of specific symptoms associated with pulmonary hypertension, that can be ameliorated by Treprostinil, recommended dosage amounts for subjects suffering from pulmonary hypertension and instructions on coordination of individual's breathing and actuation of the metered dose inhaler.

The present invention can be illustrated in more detail by the following example, however, it should be understood that the present invention is not limited thereto.

Example 1

Open Label Study Upon Acute Safety, Tolerability and Hemodynamic Effects of Inhaled Treprostinil Delivered in Seconds

A study was conducted of acute vasodilator challenge during right heart catheter investigation to determine the safety, tolerability and pulmonary vasodilatory potency of inhaled treprostinil applied in seconds by a soft mist inhaler (SMI-TRE). The study produced evidence for a long lasting

favourable effect of SMI-TRE on pulmonary hemodynamics in absence of systemic side effects and gas exchange disruptions.

Summary:

Inhaled nitric oxide (20 ppm; n=45) and inhaled treprostinil sodium (TRE; n=41) or placebo (n=4) were applied once during right heart catheter investigation. TRE was delivered in 2 breaths (1000 μg/ml aerosol concentration; 30 μg dose; n=12), 3 breaths (1000 μg/ml; 45 μg; n=9) or 2 breaths (2000 μg/ml; 60 μg; n=20) from a Respimat® SMI. Pulmonary hemodynamics and blood gases were measured at defined time points, observation time following TRE application was 120 minutes. TRE doses of 30 μg, 45 μg and 60 μg reduced pulmonary vascular resistance (PVR) to 84.4±8.7%, 71.4±17.5% and 77.5±7.2% of baseline values, respectively (mean±95% confidence interval). The 120 minute area under the curve for PVR for placebo, 30 μg, 45 μg and 60 μg TRE was 1230±1310, −870±940, −2450±2070 and −2000±900 min %, respectively. Reduction of PVR by a single inhalation of the two higher doses outlasted the observation period of 120 minutes. Reduction of systemic vascular resistance and pressure was negligible, showing a high pulmonary selectivity for SMI-TRE. Intrapulmonary selectivity was also provided by SMI-TRE as ventilation/perfusion matching, assessed by the multiple inert gas elimination technique in 5 patients with gas exchange problems, was not significantly different after SMI-TRE compared to inhaled nitric oxide or no treatment. No significant side effects were observed.

Conclusions: The acute application of inhaled treprostinil with a metered dose inhaler in 2-3 breaths was safe, well tolerated and induced a strong and sustained pulmonary selective vasodilation.

Methods and Patients

A total number of 45 patients with moderate to severe precapillary pulmonary hypertension were enrolled. Patient characteristics were: female to male ratio (f/m)=29/16, age 59±2.3 years, pulmonary artery pressure (PAP) 45±1.8 mmHg, pulmonary vascular resistance (PVR) 743±52 dynes·s·cm^{-5}, pulmonary artery wedge pressure (PAWP) 8.6±0.5 mmHg, central venous pressure (CVP) 6.4±0.7 mmHg, cardiac output (CO) 4.5±0.2 l/min, central venous oxygen saturation (SvO2) 62.3±1.2 mmHg (mean±Standard Error of the Mean). Disease etiologies were idiopathic PAH (iPAH) (n=13), PAH other (n=11), chronic thromboembolic pulmonary hypertension (CTEPH) (n=17) and pulmonary fibrosis (n=4). Table 1 presents the patient characteristics of the different groups.

TABLE 1

Patient characteristics of the different treatment groups. Data are given as mean ± Standard Error of the Mean (SEM).

	Placebo (n = 4)	30 μg TRE (n = 12)	45 μg TRE (n = 9)	60 μg TRE (n = 20)
Age [years]	61 ± 8	53.9 ± 3.9	54.2 ± 5.7	65.5 ± 3.1
PAP [mmHg]	49.5 ± 10.1	45 ± 3.1	54.3 ± 2.8	39.7 ± 2.0
PVR [Dynes]	896 ± 163	597 ± 53.9	1049 ± 107	663 ± 81
CO [l/min]	4.46 ± 0.9	5.2 ± 0.4	3.9 ± 0.4	4.4 ± 0.3
SAP [mmHg]	98 ± 8.1	90.1 ± 3.2	82.8 ± 3.9	86.1 ± 2.0
SaO2 [%]	85.3 ± 4.5	90.0 ± 1.1	89.6 ± 1.1	90.6 ± 0.5
SvO2 [%]	57.5 ± 3.9	66.0 ± 1.6	59.1 ± 3.4	62.5 ± 1.6

PAP = pulmonary artery pressure; PVR = pulmonary vascular resistance; CO = cardiac output; SAP = systemic arterial pressure; SaO2 = arterial oxygen saturation; SvO2 = central venous oxygen saturation.

Baseline values were determined 20-30 minutes after placement of the catheter. Heart rate, pulmonary and systemic blood pressure and cardiac output were measured and blood gases were taken during each pharmacological intervention at defined time points. Pharmacological interventions included the inhalation of 20 ppm nitric oxide (NO) after evaluation of baseline parameters (n=45) and the consecutive inhalation of placebo (n=4), 30 μg SMI-TRE (n=12), 45 μg SMI-TRE (n=9) or 60 μg (n=20) SMI-TRE. Placebo and treprostinil was applied with the Respimat® SMI. For filling of this device with treprostinil sodium, the placebo solution was withdrawn from the device with a syringe and treprostinil solution was injected into the device under sterile conditions. Aerosol quality was controlled before and after refilling of the SMI devices by laser diffractometry, see e.g. Gessler T., Schmehl T., Hoeper M. M., Rose F., Ghofrani H. A., Olschewski H. et al. Ultrasonic versus jet nebulization of iloprost in severe pulmonary hypertension. Eur. Respir. J. 2001; 17:14-19 incorporated herein in its entirety. The aerosol sizes before (placebo) and after filling (treprostinil) were unchanged. The aerosol particles mass median aerodynamic diameter of treprostinil-aerosol was 4-5 μm, which can be at the upper limit for alveolar deposition. The aerosol volume delivered by one cycle from the SMI was 15 μl. The solution used for aerosol generation was prepared from treprostinil sodium salt using a standard protocol. The SMI was either filled with a concentration of 1000 μg/ml treprostinil sodium (one aerosol puff=15 μg TRE) or with 2000 μg/ml (one puff=30 μg TRE). The different doses were applied as 2 puffs 1000 μg/ml (30 μg), 3 puffs 1000 μg/ml (45 μg) and 2 puffs 2000 μg/ml (60 μg). The placebo was inhaled as 2 puffs from a placebo-SMI. Hemodynamics and gas-exchange parameters were recorded for 120 minutes after TRE inhalation. This study used the Respimat® device, because the implemented "soft mist" technology was well suited for the deposition of such highly active drugs like prostanoids.

The impact of SMI-TRE on ventilation-perfusion matching was assessed in five patients (30 μg TRE, n=2; 45 μg TRE, n=1; 60 μg TRE, n=2) with pre-existing gas exchange problems by use of the multiple inert gas elimination technique (MIGET), see e.g. Wagner P D, Saltzman H A, West J B. Measurement of continuous distributions of ventilation-perfusion ratios: theory. J Appl Physiol. 1974; 36:588-99; Ghofrani H A, Wiedemann R, Rose F, Schermuly R T, Olschewski H, Weissmann N et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. Lancet. 2002; 360:895-900, both incorporated herein in their entirety.

Statistics:

Mean values, standard deviation, standard error of the mean and 95% confidence intervals were calculated. Statistical analysis was done by use of a paired t-test.

Results:

The inhalation of treprostinil sodium from the metered dose inhaler (SMI-TRE) was well tolerated, only mild and transient cough for a maximum of one minute was reported. No systemic side effects like headache, flush, nausea or dizziness were observed.

Two to three breaths of SMI-TRE induced a strong pulmonary vasodilation that outlasted the observation time of 120 minutes (45 and 60 μg). The lower dose of 30 μg TRE induced a somewhat shorter effect on pulmonary vascular resistance; however, the maximal pulmonary vasodilation was comparable. In contrast, placebo inhalation did not induce pulmonary vasodilation. In fact a slight increase in PVR over the time of the right heart catheter investigation could be recorded following placebo inhalation (FIG. **1**). The effect of SMI-TRE on systemic vascular resistance and

pressure was very small and not clinically significant. Cardiac output was significantly increased over the whole observation period, whereas heart rate was rather unchanged. Gas exchange was not influenced by SMI-TRE (FIG. **2**). The maximal changes in hemodynamic and gas-exchange parameters compared to baseline values are depicted in Table 2.

TABLE 2

Extremes of the relative changes of hemodynamic and gas exchange parameters compared to baseline after inhalation of Placebo (n = 4), 30 μg treprostinil (n = 12), 45 μg treprostinil (n = 9) and 60 μg treprostinil (n = 20). Highest (max) and lowest (min) values during the observation period are shown. Data are given as percent of baseline values (mean ± SEM).

	Placebo	30 μg TRE	45 μg TRE	60 μg TRE
PAP (min)	99.4 ± 3.0	83.4 ± 3.2	77.6 ± 6.8	79.5 ± 2.4
PVR (min)	101.4 ± 1.9	84.4 ± 4.4	71.4 ± 8.9	77.5 ± 3.7
CO (max)	99.7 ± 1.1	108.8 ± 3.8	108.6 ± 5.6	103.8 ± 2.0
SVR (min)	104.3 ± 4.3	97.7 ± 4.2	92 ± 3.9	91.3 ± 2.1
SAP (min)	102.7 ± 1.7	97.3 ± 1.9	96.1 ± 1.5	93.6 ± 2.9
HR (max)	105 ± 2.1	106.1 ± 2.9	99.1 ± 2.4	101.1 ± 0.9
SaO2 (min)	98.2 ± 0.4	101 ± 0.3	94.4 ± 1.8	95.8 ± 0.9
SvO2 (max)	104.5 ± 1.4	102.4 ± 1.3	104.5 ± 4.4	102 ± 1.0

PAP = pulmonary artery pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance; CO = cardiac output; SAP = systemic arterial pressure; HR = heart rate; SaO2 = arterial oxygen saturation; SvO2 = central venous oxygen saturation.

The areas under the curve for PVR were calculated for placebo and the different SMI-TRE doses over the 120 minute observation period (FIG. 3). A dose effect of SMI-TRE with a trend to a more sustained effect with the two highest doses could be observed.

The inhalation of a highly concentrated aerosol can be in theory prone to disturbances of gas exchange because the deposition of even small amounts of aerosol may deliver high doses locally and thereby antagonize the hypoxic pulmonary vasoconstriction in poorly ventilated areas. This would then lead to increased shunt flow or increase of low ventilation/perfusion (V/Q) areas. This question was addressed in five patients with the multiple inert gas elimination technique (MIGET), the gold-standard for intrapulmonary V/Q ratio determination. The MIGET patients were selected for pre-existing gas exchange limitations. Characteristics of these patients were: PAP 54.6±3.2 mmHg, PVR 892±88 dynes, SaO2 91.7±0.5%, SvO2 65.2±1.8%. Etiologies were iPAH (n=1), CTEPH (n=3), pulmonary fibrosis (n=1). The maximal relative reduction of SaO2 after inhalation of SMI-TRE in these patients was −3.8±1.5% compared to baseline values. Shunt flow at baseline, NO-inhalation and 60 minutes after SMI-TRE was 6.4±4.3%, 5.4±3.0% and 8.3±3.4%, respectively (mean±95% confidence interval; FIG. **4**).

No significant increase in low V/Q areas or shunt fraction after inhalation of SMI-TRE was observed, in fact the distribution of perfusion was not different to that at baseline and during nitric oxide inhalation. This proves an excellent intrapulmonary selectivity of SMI-TRE, which is also reflected by unchanged arterial oxygen saturation.

Conclusion:

Treprostinil is tolerated at high doses with no systemic side effects. The application of an effective amount of treprostinil in only few or even one single breath was achieved with a highly concentrated treprostinil sodium solution. Treprostinil can be applied by a metered dose inhaler, such as Respimat® soft mist inhaler.

Example 2

Investigation of the Effects of Inhaled Treprostinil on Pulmonary Hemodynamics and Gas Exchange in Severe Pulmonary Hypertension

This study investigated the effects of inhaled treprostinil on pulmonary vascular resistance in severe pulmonary hypertension and addressed systemic effects and gas exchange as well as tolerability and efficacy of high doses of treprostinil given in short time. A total of 123 patients with a mean pulmonary artery pressure of about 50 mmHg were investigated in three separate randomized studies. Inhaled treprostinil exerted potent sustained pulmonary vasodilation with excellent tolerability and could be safely applied in a few breaths or even one breath.

Summary:

Three different studies were conducted on a total of 123 patients by means of right heart catheterization: i) a randomized crossover-design study (44 patients), ii) a dose escalation study (31 patients) and iii) a study of reduction of inhalation time while keeping the dose fixed (48 patients). The primary endpoint was the change in pulmonary vascular resistance (PVR).

The mean pulmonary artery pressure of the enrolled patients was about 50 mmHg. Hemodynamics and patient characteristics were similar in all studies. In study i) TRE and Iloprost (ILO), at an inhaled dose of 7.5 μg, displayed comparable PVR decrease, with a significantly different time course ($p<0.001$), TRE exhibiting a more sustained effect on PVR ($p<0.0001$) and less systemic side effects. In study ii) placebo, 30 μg, 60 μg, 90 μg or 120 μg TRE were applied with drug effects being observed for 3 hours after inhalation. A near-maximal acute PVR decrease was observed at 30 μg TRE. In study iii) TRE was inhaled with a pulsed ultrasonic nebulizer, mimicking a metered dose inhaler. 15 μg TRE was inhaled with 18 pulses (TRE concentration 100 μg/ml), 9 pulses (200 μg/ml), 3 pulses (600 μg/ml), 2 pulses (1000 μg/ml) or 1 pulse (2000 μg/ml), each mode achieving comparable, sustained pulmonary vasodilation.

Inhaled treprostinil exerts sustained pulmonary vasodilation with excellent tolerability at doses, which may be inhaled in a few or even one breath. Inhaled treprostinil is advantageous to inhaled iloprost in terms of duration of effect and systemic side effects. Inhaled treprostinil is well tolerated in concentrations up to 2000 mg/ml (bringing down inhalation time to a single breath) and in high doses (up to 90 μg).

Methods:

All inhalations were performed with the OPTINEB® ultrasonic nebulizer (Nebutec, Elsenfeld, Germany).

Study i) was a randomized, open-label, single-blind cross-over study. The primary objective was to compare the acute hemodynamic effects and the systemic side effects of inhaled treprostinil with inhaled iloprost at comparable doses. A total number of 44 patients with moderate to severe precapillary pulmonary hypertension were enrolled. Patient characteristics and hemodynamic as well as gas exchange parameters are outlined in Table 3.

TABLE 3

Patient characteristics, hemodynamic parameters and gas exchange values at baseline, before challenge with inhalative prostanoids.

	N	Age	Gender f/m	Etiology i/o/t/f	PAP [mmHg]	PVR [dyn * s * cm^{-5}]	SAP [mmHg]	CVP [mmHg]	PAWP [mmHg]	CO [l/min]	SaO2 [%]	SvO2 [%]
1a	14	55.1 ± 4.8	11/3	4/4/2/4	53.8 ± 3.1	911 ± 102	95.4 ± 3.6	7.4 ± 1	8.0 ± 0.8	4.3 ± 0.4	93.8 ± 2	63.9 ± 2.4
1b	14	54.1 ± 3.3	10/4	1/6/5/2	47.4 ± 3.8	716 ± 80	90.6 ± 3.3	5.9 ± 1.4	6.4 ± 0.7	4.7 ± 0.4	92 ± 1	64.4 ± 2.3
1c	16	56 ± 2.9	7/9	6/3/6/1	47.5 ± 4.5	777 ± 102	92 ± 4.5	8.3 ± 1.4	8.6 ± 1.4	4.4 ± 0.5	91.4 ± 0.9	59.8 ± 2.6
2a	8	60.8 ± 4	4/4	2/2/3/1	51.9 ± 4.9	849 ± 152	95.9 ± 4.8	7.6 ± 1.4	11.1 ± 1.7	4.4 ± 0.6	89.6 ± 2.8	60.1 ± 2.8
2b	8	52.8 ± 6.6	6/2	1/3/3/1	49 ± 4	902 ± 189	92.4 ± 2.4	4.8 ± 1.1	7.2 ± 1.3	4.0 ± 0.4	92.4 ± 2.4	62.5 ± 1.7
2c	6	56.8 ± 5.9	4/2	0/2/2/2	44.2 ± 3.5	856 ± 123	96.3 ± 3.9	5 ± 1.1	6 ± 1	3.8 ± 0.3	92.8 ± 1.5	63.6 ± 1.8
2d	6	51.2 ± 3.8	4/2	2/2/2/0	55.5 ± 4.9	940 ± 110	91.2 ± 8.1	11.2 ± 1.2	10 ± 0.7	3.9 ± 0.4	92 ± 1.9	62 ± 5.8
2e	3	57.3 ± 9.1	1/2	0/1/0/2	45.3 ± 5.2	769 ± 267	99 ± 3.2	5 ± 2.1	9 ± 0.6	4.5 ± 0.6	94.2 ± 1.3	66.3 ± 1.5
3a	6	52.7 ± 6.6	4/2	2/4/0/0	53.8 ± 6.7	928 ± 145	92.7 ± 7.9	8.7 ± 2.7	8.8 ± 1.3	4.2 ± 0.6	90.4 ± 2.8	64.8 ± 4.3
3b	6	58.3 ± 3.5	4/2	3/1/1/1	54.2 ± 6.1	808 ± 156	94.3 ± 2.8	7 ± 1.4	10 ± 1.3	5 ± 0.7	91.9 ± 0.7	63.5 ± 2.9
3c	21	57.4 ± 5.6	8/3	7/7/6/1	46.1 ± 2.5	900 ± 99	88 ± 2.8	9 ± 1.4	9.2 ± 0.5	3.7 ± 0.3	91.7 ± 0.5	59.7 ± 2
3d	7	55.6 ± 5.8	3/4	0/4/3/0	53.1 ± 7.1	732 ± 123	91.4 ± 5.6	7.9 ± 3.1	8.6 ± 1.3	5 ± 0.4	90.7 ± 1.4	61.3 ± 3.7
3e	8	59 ± 5.2	7/1	0/4/4/0	45.1 ± 3.9	733 ± 114	92.8 ± 6.8	4.6 ± 0.8	8.1 ± 1.1	4.3 ± 0.2	90.7 ± 0.8	66.3 ± 2.8

Group 1 corresponds to study i); randomized crossover study comparing inhaled iloprost (ILO) and inhaled treprostinil (TRE).
a = 7.5 g ILO vs. 7.5 µg TRE,
b = 7.5 g ILO vs. 15 µg TRE (6 min inhalation time),
c = 7.5 g ILO vs. 15 µg TRE (3 min inhalation time).
Group 2 corresponds to study ii); evaluation of maximal tolerated dose of TRE.
a = placebo inhalation,
b = 30 µg TRE,
c = 60 µg TRE,
d = 90 µg TRE,
e = 120 µg TRE.
Group 3 corresponds to study iii); reduction of inhalation time by increase of TRE concentration, aiming at a total inhaled dose of 15 µg.
a = 18 pulses of 100 µg/ml TRE,
b = 9 pulses of 200 µg/ml TRE,
c = 3 pulses of 600 µg/ml TRE,
d = 2 pulses of 1000 µg/ml TRE,
e = 1 pulse 2000 µg/ml TRE.
Etiology of pulmonary hypertension was classified as idiopathic PAH (i), PAH of other causes (o), chronic thromboembolic PH (t), and pulmonary fibrosis (f).

Each patient inhaled both iloprost and treprostinil on the same day during right heart catheter investigation; the drugs were administered consecutively with a one hour interval between the drug applications. One half of the study patients initially inhaled treprostinil and then inhaled iloprost (n=22), while the other half initially inhaled iloprost and then inhaled treprostinil (n=22). Patients were randomized to one of the two groups and blinded as to the study drugs. Drug effects were monitored for 60 minutes after each inhalation. Iloprost was inhaled at 4 µg/ml (6 min inhalation time; n=44) and treprostinil was inhaled at a concentration of 4 µg/ml (6 min inhalation; n=14), 8 µg/ml (6 min inhalation; n=14) or 16 µg/ml (3 min inhalation; n=16). Based on previous biophysical characterization of the ultrasonic device with iloprost- and treprostinil-solution, this corresponds to a total inhaled dose of 7.5 µg iloprost and treprostinil (4 µg/ml) and 15 µg treprostinil (8 µg/ml and 16 µg/ml), respectively.

Study ii) was a randomized, open-label, single blind, placebo controlled study. The primary objectives were to describe the pharmacodynamic and pharmacokinetic effects of inhaled treprostinil at a well tolerated dose (30 µg) and to explore the highest tolerated single dose. A total number of 31 patients inhaled either placebo or treprostinil; each patient received one inhalation. The first 16 patients were randomized to 30 µg TRE (16 µg/ml, n=8) or placebo (stock solution in a concentration corresponding to TRE 16 µg/ml). Subsequent patients received 60 µg TRE (32 µg/ml; n=6), 90 µg TRE (48 µg/ml; n=6) and 120 µg TRE (64 µg/ml; n=3). Inhalation time was 6 minutes in all groups. Hemodynamics and gas-exchange as well as arterial treprostinil concentrations were recorded for 180 minutes.

Study iii) was a randomized, open-label, single blind study. The primary objective was to explore the shortest possible inhalation time for a 15 µg dose of inhaled treprostinil. A total of 48 patients inhaled one dose of TRE during right heart catheter investigation. The drug was applied in 18, 9, 3, 2 or 1 breaths. The aerosol was generated by a pulsed ultrasonic nebulizer (OPTINEB®, Nebutec, Elsenfeld, Germany) in cycles consisting of 2 seconds aerosol production (pulse) and 4 seconds pause. The device included an opto-acoustical trigger for the patient to synchronize the inspiration to the end of the aerosol pulse, thereby providing exact dosage. The TRE dose of 15 µg was either generated during 18 cycles (OPTINEB® filled with 100 µg/ml TRE, n=6), 9 cycles (200 µg/ml TRE, n=6), 3 cycles (600 µg/ml TRE, n=21), 2 cycles (1000 µg/ml TRE, n=7) or 1 cycle (2000 µg/ml TRE, n=8). Hemodynamics and gas exchange were recorded for 120-180 minutes.

Treprostinil plasma concentrations were assessed in study ii) at 10, 15, 30, 60 and 120 minutes after inhalation. Treprostinil quantification was done by Alta Analytical Laboratory (El Dorado Hills, Calif., USA) with a validated liquid chromatography atmospheric-pressure ionization tandem mass spectrometry as previously described Wade M., et al. J. Clin. Pharmacol. 2004; 44:503-9. Mixed venous blood was drawn at the depicted time points (FIG. **11**) after inhalation, centrifuged and the plasma frozen at −80° C. until temperature controlled shipping on dry ice.

Statistics:

For statistical analysis of study i) the repeated PVR measurements after inhaled iloprost and treprostinil were subjected to a three-factorial analysis of variance (ANOVA; factors: time (A), drug (B), treprostinil concentration (C)) to avoid multiple testing. The time to maximum PVR decrease after inhalation of iloprost versus treprostinil was compared by paired t-test. Area under the curve (AUC) was calculated from start of inhalation until 60 min after inhalation. Means, standard error of the mean (SEM) and 95% confidence

intervals were calculated. For study ii) and iii) areas between curves (ABC) were calculated between placebo inhalation (study ii) and the respective treprostinil inhalation until 180 min (study ii)) and 120 min (study iii)) after end of inhalation.

Results:

The inhalation of iloprost as well as treprostinil in study i) resulted in a rapid decrease in PVR and PAP (FIG. **5-7**). No significant differences were observed for the areas under the curve (AUC) of PVR decrease after inhalation of 7.5 μg TRE in 6 minutes (AUC −12.6±7.0%), 15 μg TRE in 6 minutes (AUC −13.3±3.2%) and 15 μg TRE in 3 minutes (AUC −13.6±4.3%). The AUC for PVR after the inhalation of 7.5 μg iloprost in 6 minutes was −7.7±3.7% (mean±95% confidence interval). An overview of the pooled data of treprostinil inhalation as compared to iloprost inhalation is given in FIG. **7**. The maximum effect of iloprost and treprostinil on PVR was comparable but this effect was reached significantly later after treprostinil inhalation (18±2 min) compared to iloprost (8±1 min; mean±SEM, $p<0.0001$) and lasted considerably longer (after 60 min, PVR values in the treprostinil group had not yet returned to baseline). The increase in cardiac output was less acute but prolonged after treprostinil inhalation. Systemic arterial pressure (SAP) was unaffected by treprostinil inhalation, whereas a transient decrease was observed after iloprost inhalation. Iloprost and treprostinil did not affect gas exchange. Three-factorial ANOVA for PVR demonstrated a significant difference between repeated measurements after inhalation ($p_{(A)}<0.0001$), no significant difference between drugs ($p_B=0.1$), no difference between treprostinil concentrations ($p_{(C)}=0.74$) and a significant drug×time interaction ($p_{(A\times B)}<0.0001$). This translates into a significant effect of both drugs on PVR with comparable drug potency but a prolonged drug effect of treprostinil compared to iloprost.

In this study the occasionally observed mild side effects of iloprost inhalation at the given dose (transient flush, headache) were not observed with inhaled treprostinil. Bad taste was reported by most of the patients after inhalation of TRE. This was later found to be attributable to the metacresol preservative contained in the treprostinil solution.

In study ii) pharmacodynamics of inhaled placebo or treprostinil were observed for 180 minutes. Placebo inhalation was followed by a gradual increase in PVR over the entire observation time. Due to reduced patient numbers in the 120 μg TRE group (because of side effects, see below), the hemodynamic values for this group were not included in the graphs of this study (FIG. **8-9**). All TRE doses lead to comparable maximal decreases of PVR to 76.5±4.7% (30 μg), 73.7±5.8% (60 μg), 73.3±4.3% (90 μg) and 65.4±4.1% (120 μg) of baseline values. An extended duration of pulmonary vasodilation was noted, surpassing the 3 hour observation period for the 60 μg and 90 μg (and 120 μg) TRE doses, whereas in the 30 μg dose group the hemodynamic changes had just returned to baseline within this period. Even at the highest doses, TRE had only minor effects on systemic arterial pressure (FIG. **8**). Cardiac output was increased to a maximum of 106.8±3.2% (30 μg), 122.9±4.3% (60 μg), 114.3±4.8% (90 μg) and 111.3±3.9% (120 μg TRE). The areas between the response curves after placebo versus TRE inhalation were calculated for PVR, PAP, SVR and SAP (FIG. **9**). Areas between the curves for PVR were not significantly different for 30 μg, 60 μg and 90 μg TRE, a nearly maximal effect on PVR was already observed with 30 μg TRE. Effects on PAP and SAP were small and did not show a dose-response relationship. Gas exchange was not affected at doses up to 90 μg TRE, but arterial oxygen saturation was significantly decreased at a dose of 120 μg TRE in all 3 patients. Further dose increments were omitted due to this side effect and severe headache in one patient.

Again, bad taste of the TRE aerosol was reported by most patients. Other side effects were flushing (n=1; 30 μg TRE), mild transient cough (n=3; 60 μg TRE), mild transient bronchoconstriction that resolved after one inhalation of fenoterol (n=1; 30 μg TRE), moderate bronchoconstriction that resolved after one inhalation of fenoterol (n=1; 120 μg TRE), and severe headache (n=1; 120 μg TRE). The bad taste, the bronchoconstriction and the drop in SaO2 was attributed to metacresol in the original TRE solution. With the use of a metacresol-free solution of TRE (University Hospital Giessen, Germany; produced according to the manufacturer's protocol) in the following study, these side effects did no longer occur.

Study iii) was performed with metacresol-free TRE solution, having no specific taste and smell. A total of 48 patients were enrolled. This study aimed at the reduction of inhalation time and aerosol volume needed for pulmonary drug delivery. A modified OPTINEB® inhalation device was programmed to produce a constant amount of aerosol during repeatable pulses of aerosol generation. With this device, treprostinil could be safely utilized up to a concentration of 2000 μg/ml without considerable side effects. No relationship of number or type of side effects to TRE concentration was observed. Reported side effects were mild transient cough (n=6), mild headache (n=2) and mild jaw pain (n=1).

The reduction of PVR and PAP was comparable between all groups (FIG. **10**). TRE inhalation reduced PVR to 76.3±5.6% (18 pulses, 100 μg/ml), 72.9±4.9% (9 pulses, 200 μg/ml), 71.2±6.0% (3 pulses, 600 μg/ml), 77.4±4.5% (2 pulses, 1000 μg/ml) and 80.3±5.2% (1 pulse, 2000 μg/ml). PAP was reduced to 84.2±4.5% (18 pulses, 100 μg/ml), 84.2±4.1% (9 pulses, 200 μg/ml), 81.1±4.1% (3 pulses, 600 μg/ml), 86±4% (2 pulses, 1000 μg/ml) and 88±5.4% (1 pulse, 2000 μg/ml). Cardiac output was moderately increased in all groups, whereas systemic arterial pressure was not significantly affected.

The areas between the curves (ABC) for changes in hemodynamic and gas-exchange parameters after inhalation of 15 μg TRE versus placebo were calculated for an observation time of 120 minutes (FIG. **11**). The ABC for both PVR and PAP was comparable between all groups.

Pharmakokinetic results from study ii): Peak plasma concentrations of treprostinil were found 10-15 minutes after inhalation. Maximal treprostinil plasma concentrations (C_{max}) for the 30 μg, 60 μg, 90 μg and 120 μg doses were 0.65±0.28 ng/ml (n=4), 1.59±0.17 ng/ml (n=4), 1.74 ng/ml (n=1) and 3.51±1.04 ng/ml (n=2), respectively (mean±SEM; FIG. **12**).

Discussion:

These studies investigated whether i) the acute effects of inhaled treprostinil would be comparable to or possibly advantageous over inhaled iloprost in pulmonary hypertensive patients, ii) the inhaled prostanoid dose might be increased without substantial local or systemic side effects, and iii) if the time of inhalation, which is 6-12 minutes for iloprost, could be reduced significantly by increasing the concentration of treprostinil aerosol.

The patient population in these studies included different forms of precapillary pulmonary hypertension. All these patients had a need for therapy of pulmonary hypertension and reflected the typical population of a pulmonary hyper-

tension center. No major differences in patient characteristics or hemodynamic baseline values existed between the different groups (table 3).

In study i) it was shown that the inhalation of treprostinil and iloprost in similar doses resulted in a comparable maximum pulmonary vasodilatory effect. However, marked differences in the response profile were noted. The onset of the pulmonary vasodilatory effect of inhaled treprostinil was delayed compared to iloprost, but lasted considerably longer, with the PVR decrease continuing beyond the one-hour observation period. Although the average dose of treprostinil was higher than the iloprost dose, no systemic effects were noted after treprostinil inhalation, whereas flush and transient SAP decrease, accompanied by more prominent cardiac output increase, occurred after iloprost inhalation. Such side effects were more prominent than in previous studies with inhaled iloprost. This may have been caused by the fact that the iloprost dose used in this study was 50% higher than the recommended single inhalation dose (5 μg) and that the preceding treprostinil inhalation may have added to the systemic side effects caused by the iloprost inhalation. Surprisingly, with TRE there was no such systemic side effect, although the average effect on PVR was as potent as with iloprost.

This study used a cross-over design in order to minimize the effects of inter-individual differences in response to prostanoids. The short observation period of 1 hour was used to avoid an uncomfortably long catheter investigation. As a study limitation, the short observation interval may have caused carryover effects of the first to the second period as suggested by FIG. **5**. However, this still allowed for the interpretation of the study, that both drugs are potent pulmonary vasodilators and that treprostinil effects are significantly sustained compared to the iloprost effects.

The longer duration of action and the virtual absence of side effects (except the bitter taste of treprostinil aerosol, later attributed to metacresol) encouraged increasing the applied treprostinil dose in study ii). Observation time was extended to 3 hours to obtain precise pharmacodynamic data. Inhaled treprostinil resulted in a strong pulmonary vasodilation that outlasted the observation time of 3 hours when compared to placebo inhalation. Surprisingly, inhaled treprostinil was tolerated in doses up to 90 μg.

Study iii) successfully demonstrated that the inhalation time could be reduced to literally one single breath of 2000 μg/ml treprostinil solution, thereby applying a dose of 15 μg. This drug administration with a single breath induced pulmonary vasodilation for longer than 3 hours compared to placebo inhalation. Side effects were minor, of low frequency and not related to drug concentration. It was a surprising finding that such high concentrations of treprostinil were so well tolerated.

Conclusion:

Inhaled treprostinil can be applied in high doses (up to 90 μg) with a minimal inhalation time. Inhaled treprostinil exerts high pulmonary selectivity and leads to a long-lasting pulmonary vasodilation.

Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those of ordinary skill in the art that various modifications may be made to the disclosed embodiments and that such modifications are intended to be within the scope of the present invention.

All of the publications, patent applications and patents cited in this specification are incorporated herein by reference in their entirety.

What is claimed is:

1. A method of treating pulmonary hypertension comprising administering by inhalation to a human suffering from pulmonary hypertension a therapeutically effective single event dose of a formulation comprising treprostinil or a pharmaceutically acceptable salt thereof with an inhalation device, wherein the therapeutically effective single event dose comprises from 15 micrograms to 90 micrograms of treprostinil or a pharmaceutically acceptable salt thereof delivered in 1 to 3 breaths.

2. The method of claim **1**, wherein the inhalation device is a soft mist inhaler.

3. The method of claim **1**, wherein the inhalation device is a pulsed ultrasonic nebulizer.

4. The method of claim **1**, wherein the inhalation device is a dry powder inhaler.

5. The method of claim **1**, wherein the inhalation device is a pressurized metered dose inhaler.

6. The method of claim **4**, wherein the formulation is a powder.

7. The method of claim **6**, wherein the powder comprises particles less than 5 micrometers in diameter.

8. The method of claim **1**, wherein the formulation contains no metacresol.

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